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# PRODUCTION OF MAMMARY CARCINOMA IN MICE BY ESTROGENS

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When Lacassagne,[1] in 1932, induced mammary carcinoma in male mice by injections of estrone benzoate (theelin benzoate) it was apparent that an important step forward had been made in the understanding of the causes of mammary cancer. In several laboratories similar work was at once begun. This has already led to a variety of interesting findings concerning the general effects of the estrogens, and there is every prospect that more will follow.

Yet the information concerning the fundamental phenomena of the induction of mammary carcinoma in mice remains incomplete. It is known beyond doubt that estrogens produce the disease in male mice of some strains, but data are lacking to prove just how much, if any, these hormones increase the incidence of the disease in female mice. It has not been made clear that in other strains of mice no tumors result from the treatment. The influence of age and of breeding has not been clarified. The sequence of histologic changes induced in the breast by the hormone has not been fully described.

This state of affairs is to be expected, for these questions can be answered only by experiments in which large numbers of mice of inbred strains are used and the results analyzed by proper statistical methods. Since the mice survive two years or more, the accumulation of the necessary data requires some years.

Lacassagne's original brilliant experiment was done with only 5 mice, of which 3 were males and 2 females. He used a concentrated preparation of the hormone, namely, estrone benzoate suspended in sesame

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1. Lacassagne, A.: Compt. rend. Acad. d. sc. **195**:630, 1932.

oil, which gave a sustained estrogenic effect when injected subcutaneously once a week. The following year (1933) he reported a further experiment in which a total of 34 mice, belonging to four different strains, were given injections. The largest number of mice from any one strain was 12, from the R3 strain. Even though the numbers of animals were small, Lacassagne reached the important conclusion that tumors were much more easily induced in a strain with a high natural incidence of mammary cancer than in a strain with a lower natural incidence.[2] He has since reported several additional experiments without stating exactly how many mice he used in each.[3]

Loeb and collaborators,[4] in St. Louis, carrying on similar experiments, in 1936 reported their results with a total of 102 mice belonging to six different strains. The largest number of mice of any one strain given injections was 37, from strain A. Most of these mice, however, were given the estrogen in watery solution, in relatively small doses. Two years later, in 1938, the results in a series totaling 112 mice given injections of larger doses of estrogen in oil were reported from Loeb's laboratory.[5] These mice were from seven different strains, the largest single group being 34 mice from the C3H strain. They were used in a study of the changes produced in the vagina and cervix by the hormone. During the same year Loeb and associates[6] described the growth processes induced in the uterus by the injection of estrogen. In 1939, and again in 1940, these same authors[7] made further reports on the changes in the vagina, cervix and uterus produced by estrogen. In these later papers details as to the number of mice used and the number of tumors produced by the treatment were not included. In 1941, however, the St. Louis authors[8] made a detailed report of the incidence of mammary tumors in their total series of control and treated mice of the seven strains which they have been studying. The largest series of mice receiving injections was again from the C3H strain, a total of 74 female mice having received large doses of estrogen. Unfortunately, the report does not include an adequate statistical analysis of the ages of the mice

2. Lacassagne, A.: Compt. rend. Soc. de biol. **114**:427, 1933.

3. Lacassagne, A.: (*a*) Compt. rend. Soc. de biol. **115**:937, 1934; (*b*) Certain Biological Problems Relating to Cancer, Hormones, and Radiation, Philadelphia, The International Research Foundation, 1936, p. 12; (*c*) Compt. rend. Soc. de biol. **121**:607, 1936; (*d*) Am. J. Cancer **37**:414, 1939.

4. Suntzeff, V.; Burns, E. L.; Moskop, M., and Loeb, L.: Am. J. Cancer **27**:229, 1936.

5. Suntzeff, V.; Burns, E. L.; Moskop, M., and Loeb, L.: Am. J. Cancer **32**: 256, 1938.

6. Loeb, L.; Suntzeff, V., and Burns, E. L.: Am. J. Cancer **34**:413, 1938.

7. Loeb, L.; Suntzeff, V., and Burns, E. L.: Am. J. Cancer **35**:159, 1939; **38**:217, 1940.

8. Suntzeff, V.; Kirtz, M. M.; Blumenthal, H. T., and Loeb, L.: Cancer Research **1**:446, 1941.

at death, a vital factor, in our opinion, influencing the incidence of tumors. The data which are presented, however, do not prove that in females estrogen treatment raises the incidence of mammary carcinoma over that observed in breeding control mice. This is a point of great importance in determining the role that estrogens play in the development of carcinoma, and we shall discuss it at length later on with regard to our own experimental data.

A great deal of work on the effects of the administration of estrogens to mice has been done at Yale University, under the leadership of Allen and Gardner.[9] In 1937, the latter[9c] reported the results of repeated injections over a long period in three strains of mice. In the A strain, with a high tumor incidence, 35 males and 16 females were given injections; in the C3H strain, which also has a high tumor incidence, 24 males and 17 females were treated, while in the CBA, low tumor strain, 12 males and 7 females received injections. Although the Yale group has since published many descriptions of the effects of injected estrogen on various organs of the mouse, they have made no comprehensive report concerning the production of mammary carcinoma in different strains of mice. Allen,[9g] however, recently summed up their experience in the following terms: "Lacassagne's report of mammary cancer in male mice feminized by injections of estrogens has been completely confirmed. This has been done not only in strains of mice genetically cancer susceptible but also in strains of mice in which mammary cancer in the females is normally low (less than 5 per cent). It has not been possible to obtain mammary cancer in some strains which are cancer resistant, chiefly because they do not tolerate the estrogenic treatment; i. e., they die before the cancer age when on estrogen treatment which other strains tolerate."

Taylor and Waltman[10] have recently studied the histologic effects of injected estrogen on the mammary glands of female mice of the C57 and dba strains. The numbers of mice receiving injections were small, a total of 29 mice of both strains being given estrone and 24 mice of both strains being given estradiol benzoate (dihydrotheelin benzoate). No attempt was therefore made to determine the effects of the treatment

9. (*a*) Gardner, W.U.: Am. J. Anat. **59**:459, 1936. (*b*) Gardner, W.U.; Smith, G. M.; Strong, L.C., and Allen, E.: Arch. Path. **21**:504, 1936. (*c*) Gardner, W. U.: Influence of Estrogenic Hormones on Abnormal Growths, in Ward, H. B.: Some Fundamental Aspects of the Cancer Problem, Occasional Publication, American Association for the Advancement of Science, New York, Science Press, 1937, p. 67. (*d*) Gardner, W. U.; Allen, E.; Smith, G. M., and Strong, L. C.: J. A. M. A. **110**:1182, 1938. (*e*) Gardner, W. U., and Allen, E.: Yale J. Biol. & Med. **12**: 213, 1939. (*f*) Gardner, W. U.; Kirschbaum, A., and Strong, L. C.: Arch. Path. **29**:1, 1940. (*g*) Allen, E.: J. A. M. A. **114**:2107, 1940. (*h*) Allen, E., and Gardner, W. U.: Cancer Research **1**:359, 1941.

10. Taylor, H. C., Jr., and Waltman, C. A.: Arch. Surg. **40**:733, 1940.

on the incidence of tumors. The authors made interesting comparisons between their findings in the breasts of these treated mice and chronic mastitis in human beings.

## PLAN OF THE EXPERIMENT

We began the study of the manner in which estrogens affect the incidence of mammary carcinoma in mice five years ago, with the intention, among others, of finding out whether or not the hormone actually increases the incidence of the disease in females and what histologic changes it produces in the breast. Since there is still a lack of detailed reports regarding these questions, we feel the need of presenting our own experimental data as comprehensively as possible.

Our injections were begun in January 1936, with a supply of estrone benzoate secured, through Lacassagne, from his colleague Prof. A. Girard. This was the same preparation that Lacassagne had used in his original experiment. It was a suspension of the estrone benzoate in sesame oil, each cubic centimeter containing 1 mg. of the crystalline estrogen. After this supply of estrogen benzoate was exhausted, in 1938, the Schering Corporation, through Dr. Erwin Schwenk, supplied us with crystalline estron benzoate. The method of treatment that we adopted was also the same as that used by Lacassagne. Each mouse received 0.05 cc. of the preparation into the subcutaneous tissues of the back, through a long fine needle, once a week. The course of injections was begun when the mice were between 10 and 14 days of age.

In female mice subjected to such relatively large doses of estrogen, symptoms of its effects are apparent within two months. The mice begin to look sick and their abdomens enlarge. This is due to pyometra. The uterine horns become so distended with caseous material that perforation with peritonitis and death will follow unless the estrogen treatment is temporarily stopped. Figure 1 shows this picture in a mouse of the C57 strain that died after three and four-tenths months of treatment.

In the male mice one of the first external evidences of estrogen effect is the development of a scrotal hernia, a phenomenon that was carefully studied by Burrows [11] and by Gardner.[9a] It was usually well developed after two months of the treatment as we gave it. Soon thereafter protrusion of the lower part of the abdomen develops. This is due to distention of the bladder caused by obstruction of the prostatic urethra. Again, unless the treatment is temporarily suspended, death may result from rupture of the bladder or from pyonephrosis. Since the primary purpose of our experiment was to induce the maximal incidence of cancer in the mice, it was essential to avoid killing them with the estrogen before they reached old age, in which cancer develops. We therefore suspended treatment for a week or sometimes for two weeks when these dangerous signs of overdosage developed.

We lost some animals at an early age from overdosage. But on the whole our data show, we believe, that our mice survived to a fairly satisfactory age. Except for the intermissions we have mentioned, the treatment was kept up until death or until a tumor developed and the animal was killed. An exception was made in the case of the C57 mice that survived eighteen months of estrogen treatment. It was decided at this point that further injections would soon kill off the relatively small group that remained alive. Treatment was therefore stopped so that the

11. Burrows, H.: Brit. J. Surg. **23**:658, 1931.

animals would reach a more advanced age during which tumors might conceivably develop in them. As it happened, tumors did not appear in any. In general, the mice of C57 strain tolerated the estrogen treatment better than either the Paris or the Marsh mice.

We believe that it is of importance to describe the manner in which the mice were cared for, because living conditions have a profound influence on the age which the mice attain, and the latter factor affects the incidence of tumors developing in them. Our mice are kept in galvanized iron cages measuring 10 by 7 by 6.5 inches (25.5 by 18 by 16.5 cm.). The wire mesh cover contains a trough projecting down into the cage, into which the pellets of food are placed. The mice nibble the pellets through the wire mesh. Water is provided by bottles with drinking tubes projecting down through the wire mesh. The diet consists exclusively of

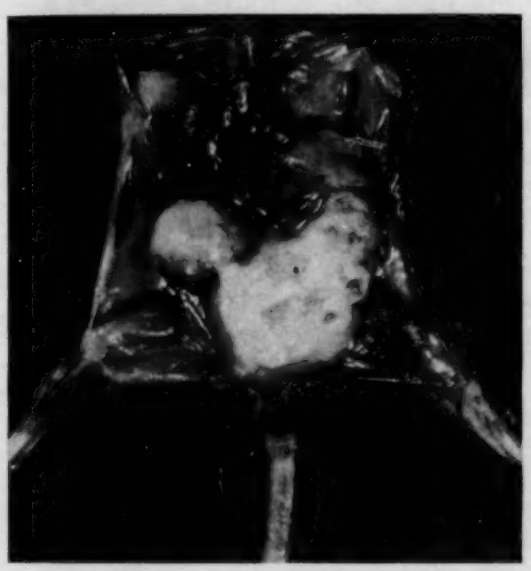

Fig. 1.—Pyometra in a mouse given injections of estrogen.

pellets manfactured for mice. The pellets contain what is presumably a complete ration. The ingredients are listed below.[12] Breeding mice are kept in separate

12. The ingredients of the Rockland mouse diet, manufactured by the Arcady Farms Milling Company, of Chicago, are:

Ground corn
Ground hulled barley
Ground hulled oats
Ground whole meat
Meat scraps
Powdered whole milk
Alfalfa leaf meal
Soybean meal
Wheat bran
Wheat midlings
Pulverized oats
Gluten feed
Fish meal
Peanut flour
Linseed
Corn oil meal
Sodium chloride (not iodized)
Precipitated chalk
Cod liver oil

pairs in these cages, while the treated mice (which did not litter) were kept 5 in each cage. The temperature in the room where the animals are kept is thermostatically controlled at between 70 and 80 F.

Early in our work we found that drastic steps to avoid epidemics of paratyphoid that sweep through mouse and rat colonies in many laboratories [13] were necessary if we were to hope to carry our mice through to old age. We therefore cultured the feces of all our animals according to a method described by Slanetz and eliminated from the colony all animals in which Salmonella enteritidis-like organisms were found. From this point on we have never admitted any new animals to our animal room, and we have not allowed those caring for other animal colonies in our institution to visit our animal room. It is a self-contained unit as regards the storage of food and the shavings used for bedding. Managed in this manner like a sterile bacterial colony our mice have had no more paratyphoid over a period of five years.

We have bred all our animals in our own animal room by brother and sister matings from three strains of mice which we selected as suitable for the experiment.

1. The Paris R3 strain from the Pasteur Laboratory of the Radium Institute at Paris. Lacassagne sent us three pairs from this strain in 1936. It was the strain with a high incidence of spontaneous mammary carcinoma that he used in his original experiment. In his laboratory 72 per cent of the strain gave tumors.
2. The Marsh strain, from the State Institute for the Study of Malignant Disease, at Buffalo. Dr. Marsh [13a] sent us ten pairs from this strain in 1933. It originated many years ago from the Lathrop-Loeb stock. At Buffalo, in 1929, from 85 to 93 per cent of the inbred females were giving tumors.
3. The C57 strain from the Roscoe B. Jackson Memorial Laboratory, at Bar Harbor, Maine. According to Little, only 1 per cent or less of this strain gave mammary carcinoma at Bar Harbor.

In view of the many factors for error in an experiment of this kind we felt that not only were control groups of untreated male and female, bred and unbred mice necessary but that the groups of control and treated animals should include at least 100 mice each. Deaths before the cancer age have reduced the numbers of our groups somewhat, but as far as possible we have adhered to this aim.

It has been our practice to kill mice in which tumors developed, as soon as the cancerous nature of the growth was apparent. Our routine autopsy has included taking sections from the tumor if there was one; from the breasts, adrenals and thyroid; from the prostate, seminal vesicles and testes of the male mouse and from the cervix, uterus and ovaries of the female mouse. Our facilities have not been such that we could carry out adequate histologic study of the pituitary in all of our animals, but this has been done in representative cases. In many instances sections were made from the femurs and the vertebrae of treated mice. Tissues have been fixed in Bouin's fluid, because it has been found that Zenker's fixative

13. Buchbinder, L.; Hall, L.; Wilens, S. L., and Slanetz, C. A.: Am. J. Hyg. **22**:199, 1935.

13a. Marsh, C. C.: J. Cancer Research **13**:313, 1929.

does not work well with the prostate and seminal vesicles of any mouse in which there has been urinary retention.

### PRESENTATION OF THE RESULTS OF THE EXPERIMENT

In the course of the experiment, which extended over a period of four years, a total of 1,526 mice surviving to the minimal cancer age were studied. Since the earliest spontaneous mammary carcinoma in our mice developed at the age of 6 months, all mice that did not attain this age in the control groups were excluded. In the treated groups the earliest induced mammary carcinoma developed at the age of 4 months; mice that did not attain this age were therefore excluded. Because of the bulk of the data that we have to present, we shall limit ourselves in the present communication to discussing the effects of injected estrogen on the breast, omitting description of the changes that are produced in other organs. These are of great interest but to present them here would be impracticable. We shall present the statistical data and the histologic observations together for each of the control and treated groups of animals, hoping in this way to correlate the incidence of mammary tumors with the actual pathologic observations in the breasts.

In comparing the findings in the various groups of mice we regard differences as *suggestive* when they approach twice the square root of the sum of the squares of the standard errors but do not reach this figure. Differences of this order are not statistically valid and may well be due to chance.

When differences are more than twice but less than three times the square root of the sum of the squares of the standard errors, we regard them as *significant*. Under these circumstances the probability that the differences are due to chance is less than 5 per cent.

For differences greater than three times the square root of the sum of the squares of the standard errors we use the expression *highly significant*. Differences of this order are due to chance in less than 1 per cent of cases.

### HISTOLOGIC OBSERVATIONS ON BREASTS OF MALE CONTROLS

*Paris Strain.*—Male mice of the Paris strain in the cancer age have little in the way of epithelial tissue in their breasts. In three quarters of our controls we found no ducts or acini at all. The microscopic appearance of such a breast is shown in figure 2 *A*. In the remaining quarter of the mice a few ducts were seen scattered throughout the fat and connective tissue of the breast, as shown in figure 2 *B*. These ducts

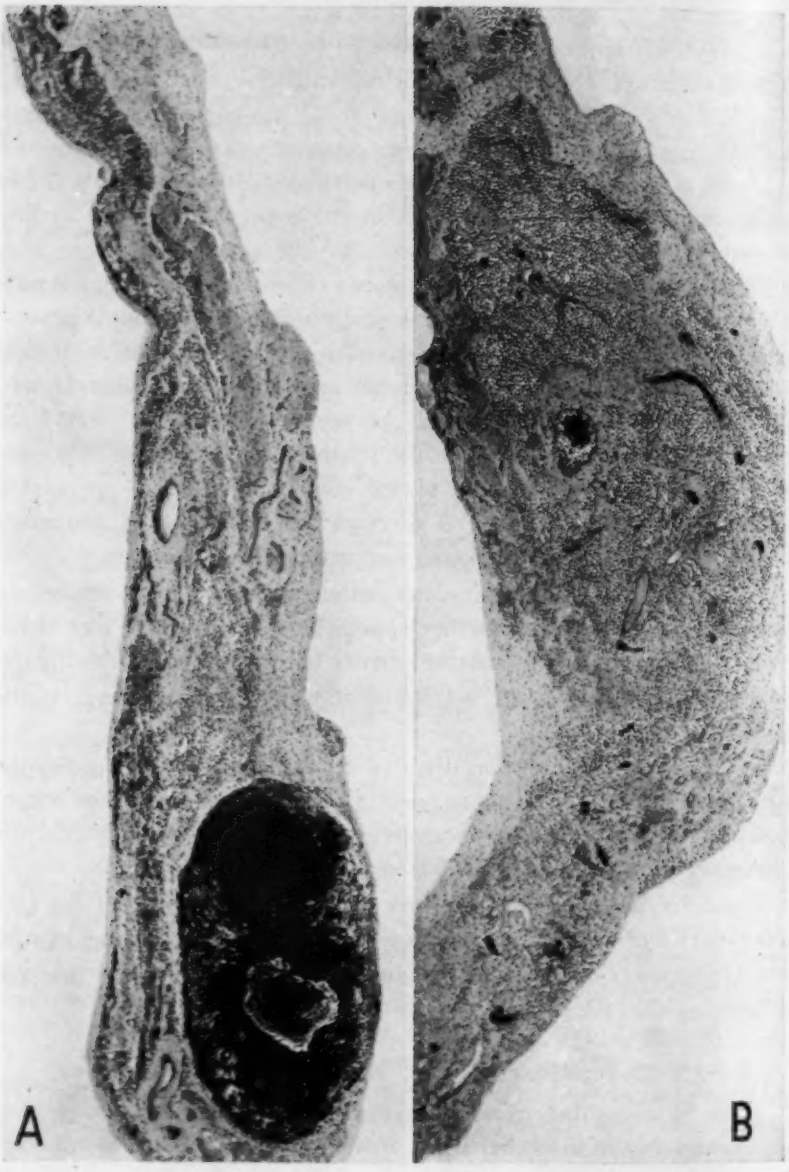


Fig. 2.—*A*, the breast as it appears in most male mice of the Paris strain. No ducts or acini are seen. The dark mass is the inguinal lymph node which was routinely dissected out with the inguinal breast. *B*, the breast as it appears in a minority of the male mice of the Paris strain. A few scattered ducts are seen.

were not surrounded by any acini. Occasionally they were elongated or slightly dilated, but in no instance did we observe intraductal proliferation of the lining epithelium. This was a single layer of low cuboid cells which did not show mitosis.

In the 2 male mice in which spontaneous mammary carcinoma developed the breasts showed scattered small ducts such as we have described, but there were no special features to distinguish them from the breasts of other control mice.

TABLE 1.—*The Male Controls*

| | Paris Strain | Marsh Strain | C57 Strain |
|---|---|---|---|
| Total group: mice surviving 6 mo..... | 141 | 51 | 96 |
| Percentage in which mammary carcinoma developed * ..... | 1.42% ± 1.00% | 0% ± 1.92% | 0% ± 1.03% |
| Mean age in mo. at death †..... | 18.86 ± 0.48 | 15.65 ± 0.59 | 22.92 ± 0.52 |
| s of age in mo. at death ‡..... | 5.68 ± 0.34 | 4.20 ± 0.42 | 5.10 ± 0.37 |
| Median age in mo. at death §..... | 20.00 ± 0.60 | 16.30 ± 0.74 | 23.40 ± 0.65 |
| Mice in which carcinoma developed..... | 2 | 0 | 0 |
| Mean age in mo. at death..... | 16.65 ± 4.60 | | |
| s of age in mo. at death..... | 6.50 ± 3.25 | | |
| Median age in mo. at death..... | 16.65 ± 5.75 | | |
| Mice in which carcinoma failed to develop ..... | 139 | 51 | 96 |
| Mean age in mo. at death..... | 18.89 ± 0.48 | | |
| s of age in mo. at death..... | 5.68 ± 0.34 | | |
| Median age in mo. at death..... | 20.00 ± 0.60 | | |

* Standard error for percentage = $\sqrt{\frac{P \times Q}{N}}$. P = percentage positive, Q = 100 − P, and N = number of cases.

† Standard error of mean = $\frac{s}{\sqrt{N}}$

Standard error of standard deviation = $\frac{s}{\sqrt{2N}}$

‡ Standard deviation (s) = $\sqrt{\frac{\Sigma d^2 F}{N} - (b_x)^2}$

§ Standard error of median = 1.25 standard error of mean.

*Marsh Strain.*—In the breasts of the male controls of the Marsh strain in the cancer age we found no epithelial elements at all. The breasts were composed of fat and connective tissue, containing a number of good-sized vessels.

*C57 Strain.*—The breasts of the male control mice of the C57 strain in the cancer age were likewise devoid of epithelial elements.

## HISTOLOGIC CHANGES IN BREASTS OF UNBRED FEMALE CONTROLS

*Paris Strain.*—The breasts of unbred female mice of the Paris strain in the cancer age contain an abundance of glandular elements (fig. 3). Ducts of moderate size, lined by a single layer of low cuboidal epithelium,

Fig. 3.—The breast of an unbred female mouse of the Paris strain, showing many ducts and their surrounding acini.

are scattered throughout the gland. These ducts are often surrounded by smaller ducts, or acini as it seems preferable to call them, which appear to develop by budding from the sides and ends of the larger ducts, as illustrated in figure 4. The epithelium of these budding acini often shows signs of increased vigor of growth; i. e., the cells are taller than those lining the ducts, their nuclei are larger and blacker, and vary somewhat in size. They sometimes pile up two or even three layers deep in places, and they occasionally show mitosis. This budding of the ducts

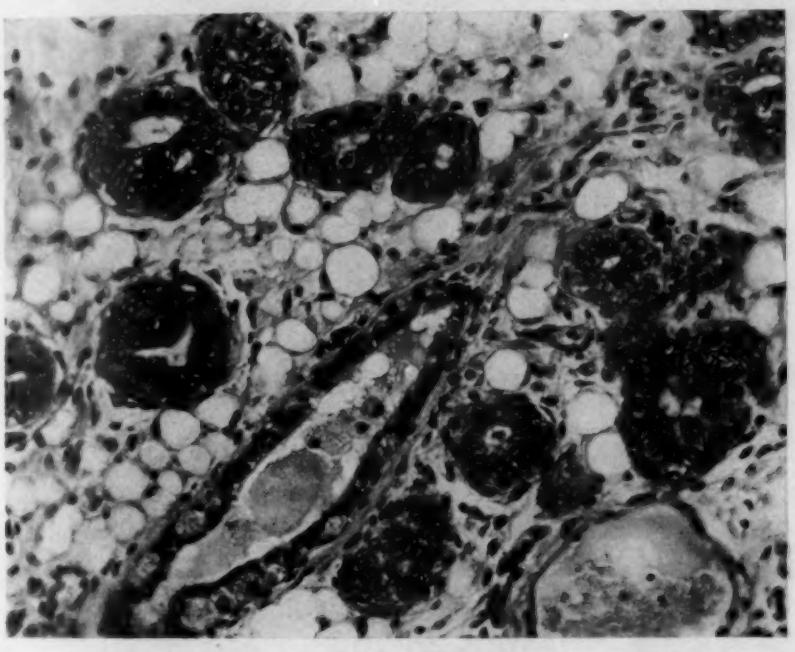

Fig. 4.—Budding of acini from a duct in the breast of an unbred female mouse of the Paris strain.

is of fundamental importance in the growth of mammary tumor in the mouse, for all stages of transition from it to fully developed carcinoma can be seen. The next stage after simple budding is the multiplication of the acini to form more or less closely packed fields like that shown in the lower part of figure 5 *A*. In such a field the lining epithelium of the acini is particularly apt to show the signs of increased vigor of growth that we have mentioned. Finally, the area of proliferation forms a more definitely circumscribed tumor, such as that seen in the upper part of

figure 5 *A*, in which the cells show all the histologic signs of undoubted malignancy, such as hyperchromatism, variation in size and shape, and frequent mitoses.

In these unbred Paris females the ducts often showed a moderate degree of dilatation. Intraductal proliferation was exceedingly rare.

Fig. 5.—*A*, multiplication of acini to form a small carcinoma in the breast of a mouse of the Paris strain. *B*, cystic dilatation of the ducts in the breast of an unbred female mouse of the Marsh strain.

*Marsh Strain.*—In unbred females of the Marsh strain in the cancer age the structure of the breast is very similar to that in the Paris mice.

There are scattered ducts lined by low cuboidal epithelium, some of which are surrounded by budding acini. Fields of proliferating acini are also seen.

Fig. 6.—*A*, the breast of an unbred female mouse of the C57 strain. *B*, the breast of a bred female mouse of the Paris strain.

Cystic dilatation is a feature of the ducts in the breasts of these mice. In more than half of our animals much dilated ducts filled up with acidophile fluid were seen (fig. 5 *B*). It is noteworthy that in only

one section was there any proliferation of the lining epithelium into the lumens of such ducts, and in this one section it was very slight in degree.

*C57 Strain.*—The epithelial elements are less prominent in the breasts of unbred female mice of the C57 strain in the cancer age than they are in the breasts of either the Paris or the Marsh unbred female mice. Figure 6 *A* shows a typical C57 breast. A number of scattered ducts are seen, lined by low cuboidal epithelium. Some of them are moderately dilated. None show intraductal proliferation.

An important point is that budding of the ducts such as we have described in the Paris and Marsh mouse breasts is practically absent. Occasionally one or two acini are seen branching from a duct, but active proliferation, in which a half dozen or a whole field of acini are formed,

TABLE 2.—*The Unbred Female Controls*

| | Paris Strain | Marsh Strain | C57 Strain |
|---|---|---|---|
| Total group: mice surviving 6 mo..... | 94 | 74 | 100 |
| Percentage in which mammary carcinoma developed ..... | 52.13% ± 5.15% | 68.92% ± 5.38% | 0% ± 0.99% |
| Mean age in mo. at death..... | 17.47 ± 0.65 | 14.06 ± 0.42 | 23.62 ± 0.62 |
| s of age in mo. at death..... | 6.29 ± 0.46 | 3.64 ± 0.30 | 6.21 ± 0.44 |
| Median age in mo. at death..... | 17.00 ± 0.81 | 13.70 ± 0.53 | 22.75 ± 0.78 |
| Mice in which carcinoma developed..... | 49 | 51 | 0 |
| Mean age in mo. at death..... | 16.44 ± 0.73 | 13.47 ± 0.40 | |
| s of age in mo. at death..... | 5.12 ± 0.52 | 2.85 ± 0.28 | |
| Median age in mo. at death..... | 16.52 ± 0.88 | 13.50 ± 0.50 | |
| Mice in which carcinoma failed to develop ..... | 45 | 23 | 100 |
| Mean age in mo. at death..... | 18.61 ± 1.07 | 15.34 ± 0.98 | |
| s of age in mo. at death..... | 7.19 ± 0.76 | 4.70 ± 0.69 | |
| Median age in mo. at death..... | 18.40 ± 1.34 | 15.20 ± 1.23 | |

does not occur in the breasts of these C57 mice. We should thus expect from the histologic observations that mammary carcinoma would not develop in them, and our statistical data bear this out.

### HISTOLOGIC CHANGES IN BREASTS OF BRED FEMALE CONTROLS

*Paris Strain.*—In bred female mice of the Paris strain in the cancer age the glandular elements in the breasts are more prominent than in unbred females. Figure 6 *B* shows a typical breast in a mouse that had had nine litters and died at the age of 14 months with a mammary carcinoma. Many small ducts, each surrounded by its several budding acini, are seen. There are a few moderately dilated ducts, without any intraductal proliferation. Several extensive fields of proliferating acini are seen, and in the middle right sector there is a sharply defined area

of closely packed acini lined by deeply hyperchromatic cells which has all the characteristics of a microscopic carcinoma.

*Marsh Strain.*—The breasts of the bred female mice of the Marsh strain in the cancer age contain somewhat more glandular tissue than

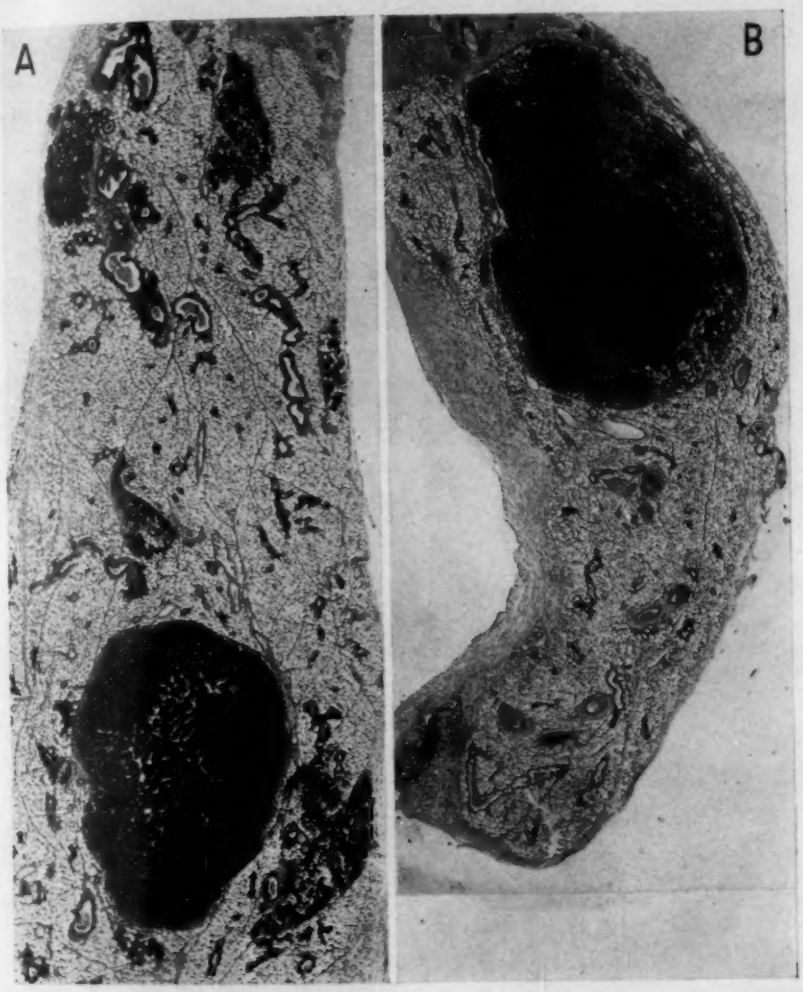


Fig. 7.—*A*, the breast of a bred female mouse of the Marsh strain. *B*, the breast of a bred female mouse of the C57 strain.

those of the unbred controls. Figure 7 *A* shows the breast of a mouse which had had three litters and died with a mammary carcinoma at the age of fourteen and eight-tenths months. It contains scattered ducts of

moderate size, which are often surrounded by a group of budding acini. Several large fields of closely packed acini are seen. As in the unbred female mice of this strain, there is a good deal of cystic dilatation of the ducts, without intraductal proliferation.

*C57 Strain.*—The breasts of bred female mice of the C57 strain in the cancer age contain much less glandular tissue than do the breasts of bred females of the Marsh and Paris strains. Scattered ducts lined by low cuboidal epithelium are seen. These ducts are not surrounded by groups of budding acini, such as are seen regularly in the breasts of mice of the Marsh and Paris strains. Some of the ducts are moderately dilated, and filled up with acidophile material. There is no intraductal proliferation.

TABLE 3.—*The Bred Female Controls*

| | Paris Strain | Marsh Strain | C57 Strain |
|---|---|---|---|
| Total group: mice surviving 6 mo..... | 140 | 55 | 93 |
| Percentage in which mammary carcinoma developed ..... | 73.57% ± 3.73% | 76.37% ± 5.73% | 1.08% ± 1.07% |
| Mean age in mo. at death..... | 12.13 ± 0.38 | 12.54 ± 0.44 | 22.25 ± 0.55 |
| s of age in mo. at death..... | 4.46 ± 0.27 | 3.29 ± 0.31 | 5.29 ± 0.39 |
| Median age in mo. at death..... | 10.90 ± 0.48 | 12.20 ± 0.55 | 23.15 ± 0.69 |
| Mice in which carcinoma developed..... | 103 | 42 | 1 |
| Mean age in mo. at death..... | 12.15 ± 0.37 | 12.77 ± 0.50 | 14.9 (1 mouse) |
| s age in mo. at death..... | 3.74 ± 0.26 | 3.21 ± 0.35 | |
| Median age in mo. at death..... | 11.10 ± 0.46 | 12.65 ± 0.63 | |
| Mice in which carcinoma failed to develop ..... | 37 | 13 | 92 |
| Mean age in mo. at death..... | 12.04 ± 0.97 | 11.81 ± 0.96 | 22.53 ± 0.55 |
| s of age in mo. at death..... | 5.88 ± 0.63 | 3.46 ± 0.68 | 5.24 ± 0.39 |
| Median age in mo. at death..... | 10.10 ± 1.21 | 10.60 ± 1.20 | 23.10 ± 0.69 |

Figure 7 *B* shows a representative breast of this kind. It is from a mouse that had eight litters and died of old age at 25.2 months. This picture is very similar to that of the breast from an unbred C57 female.

The differences between the structure of the mammary gland in these mice of a low cancer strain, and its structure in mice of a high cancer strain are perhaps best shown by comparison of cleared mounts of the whole organ. Figures 8 and 9 show such preparations of glands from unbred female mice, 6 months of age, of the C57 and Paris strains, respectively. In the C57 breast the larger ducts have only a few branches of the first order and they end in blunt tips without any buds. In the Paris breast the branches have a multitude of buds, which project in clusters from their sides and tips. These are the budding acini which we have described in an earlier section.

Fig. 8.—Cleared mount of the breast of an unbred female mouse of the C57 strain.

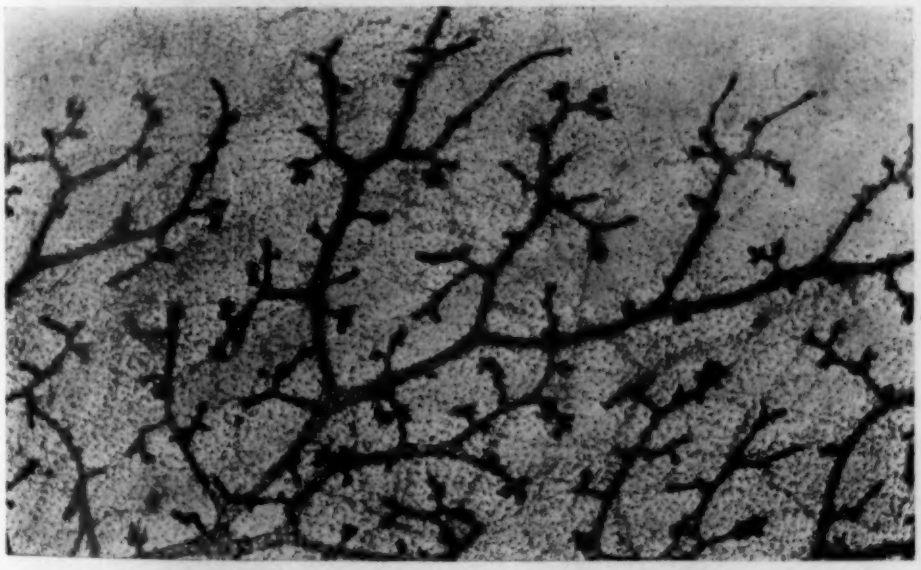

Fig. 9.—Cleared mount of the breast of an unbred female mouse of the Paris strain.

HISTOLOGIC CHANGES IN BREASTS OF ESTROGEN-TREATED MALES

*Paris Strain.*—The breasts of male mice of the Paris strain given injections of maximal doses of estrogen show intense stimulation of the epithelial elements. Figure 10 *A* shows such a breast, from a mouse

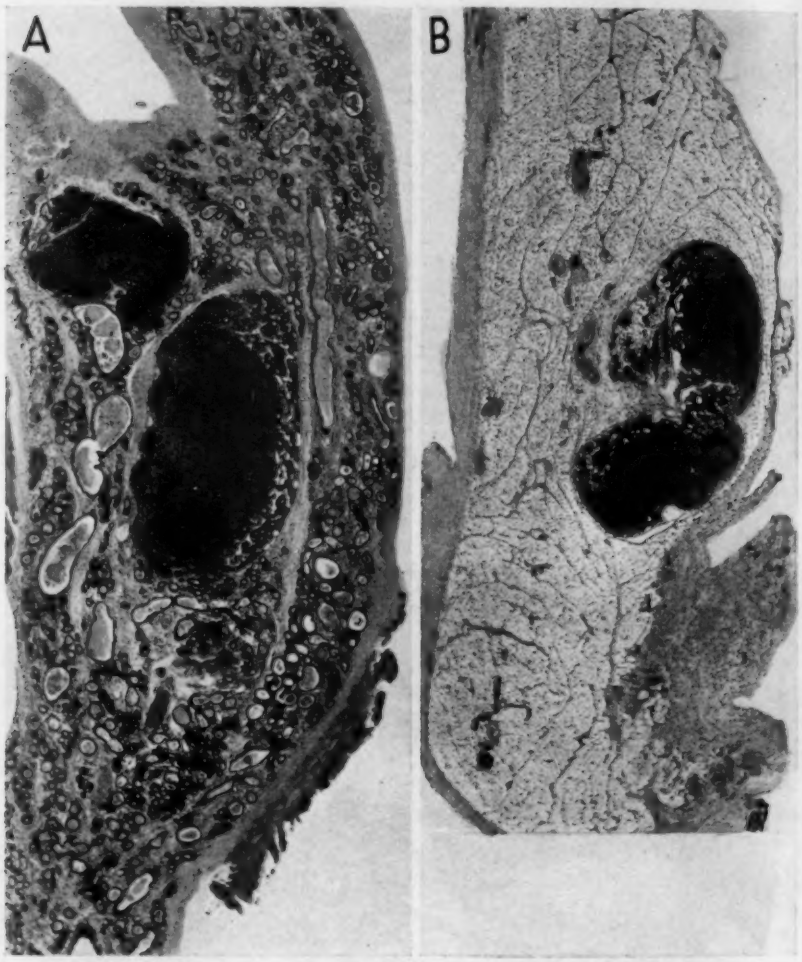


Fig. 10.—*A*, the breast of a male mouse of the Paris strain treated with estrogen. *B*, the breast as it appeared in most male mice of the Marsh strain treated with estrogen.

treated for nine and nine-tenths months which died with mammary carcinoma at two sites. The entire organ is seen to be jammed full of closely packed ducts. These are of all sizes, some being greatly dilated and filled with acidophile material, as if there had been increased secretion. They are lined with cuboidal epithelium which does not proliferate intraductally

except in rare instances. We found only 2 examples of such proliferation in our series of mice. Many of the smaller ducts are surrounded by budding acini. The cells lining these acini often show evidence of increased vigor of growth; they are larger, darker, sometimes in mitosis, and may be two layers thick in places. Broad fields of these proliferating acini are seen. Sharply defined, closely packed masses of such acini develop, which have all the histologic characteristics of carcinoma. There is such a small carcinoma in the upper left hand sector of figure 10 *A*.

We can conclude that estrogen treatment of these male Paris mice produces in their breasts even more evidence of glandular proliferation than it does in the breasts of untreated bred females of the same strain. This proliferation is the kind that is usually associated with carcinoma

TABLE 4.—*The Estrone-Treated Males*

| | Paris Strain | Marsh Strain | C57 Strain |
|---|---|---|---|
| Total group: mice surviving 4 mo..... | 112 | 82 | 104 |
| Percentage in which mammary carcinoma developed ..... | 87.50% ± 3.13% | 14.63% ± 3.90% | 0% ± 0.90% |
| Mean age in mo. at death..... | 8.11 ± 0.26 | 10.35 ± 0.41 | 12.05 ± 0.32 |
| s of age in mo. at death..... | 2.78 ± 0.19 | 3.69 ± 0.29 | 3.21 ± 0.22 |
| Median age in mo. at death..... | 7.55 ± 0.33 | 9.05 ± 0.51 | 12.85 ± 0.40 |
| Mice in which carcinoma developed..... | 98 | 12 | 0 |
| Mean age in mo. at death..... | 7.98 ± 0.22 | 12.40 ± 0.91 | |
| s of age in mo. at death..... | 2.17 ± 0.16 | 3.15 ± 0.64 | |
| Median age in mo. at death..... | 7.55 ± 0.20 | 11.80 ± 1.15 | |
| Mice in which carcinoma failed to develop ..... | 14 | 70 | 104 |
| Mean age in mo. at death..... | 9.08 ± 1.41 | 10.17 ± 0.45 | |
| s of age in mo. at death..... | 5.29 ± 1.00 | 3.76 ± 0.32 | |
| Median age in mo. at death..... | 7.35 ± 1.76 | 8.75 ± 0.56 | |

development in the mouse breast, and it is therefore not surprising that the disease appears in the great majority of these estrogen-treated males.

*Marsh Strain.*—In the great majority of the male mice of the Marsh strain given injections of maximal doses of estrogen the breasts showed no apparent change. Figure 10 *B* shows the breast from such an animal. It died after six months of treatment. The breast is seen to be entirely without epithelial elements, this despite the fact that the genital apparatus of the animal showed the characteristic estrogen changes in a marked degree. The seminal vesicles were greatly reduced in size and surrounded by a thick sheath of fibrous tissue, and their papillary epithelial infoldings were gone. The coagulating glands were dilated to form large cysts filled with squamous debris, their cuboidal epithelium having undergone squamous metaplasia. The single-layered low cuboidal epithelium of the prostate had proliferated to form several irregular layers of more hyperchromatic cells, and the glands lay in a bed of dense fibrous tissue.

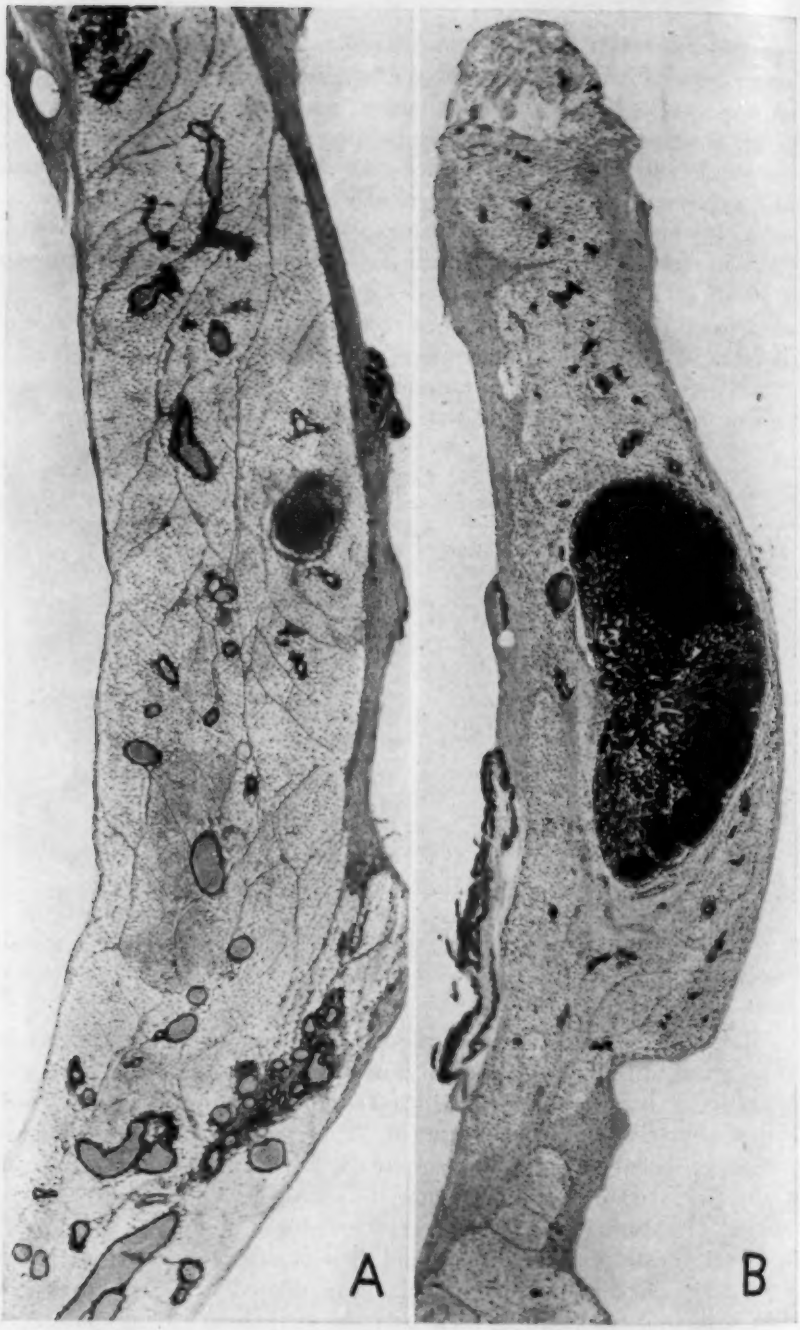


Fig. 11.—*A*, the breast as it appeared in a minority of the male mice of the Marsh strain treated with estrogen. *B*, the breast of a male mouse of the C57 strain treated with estrogen.

In a few of these Marsh mice a moderate degree of epithelial proliferation was found in one breast, as shown in figure 11 *A*. Here a number of moderately dilated ducts, a few of which are surrounded by budding acini, are seen. In two areas these budding acini form small more or less solid fields of glands. As might be expected from this histologic alteration, a carcinoma developed in this mouse after eleven and three-tenths months of injection. It is of interest that another breast from this same animal was entirely devoid of epithelial elements.

We must conclude that estrogens as a rule do not stimulate the breasts of male March mice. In some instances, however, a moderate degree of glandular proliferation is induced, and it is this kind of change that results in the occasional mammary carcinoma that the treatment produces in the males of this strain.

*C57 Strain.*—In one third of the male mice of the C57 strain treated with estrogen the breasts showed no change, but in the other two thirds scattered ducts developed. Figure 11 *B* shows the breast of such a mouse that died after seventeen months of treatment, without a mammary carcinoma. The ducts are small, and most of them show no acinous buds at all. Occasionally one or two acini are seen branching off from a duct, but larger groups of proliferating acini, which are characteristic of the breasts of the Paris strain mice and are occasionally seen in the breasts of the Marsh strain mice, are not found at all in these C57 strain mice. The ducts produced by the estrogen treatment in these mice are sometimes moderately dilated and filled up with acidophil material. Intraductal proliferation was found in only 1 mouse in our series.

We can conclude that vigorous estrogen treatment of these C57 male mice often causes elements of the duct system, presumably lying dormant in the breast, to appear. But it does not produce the kind of acinous proliferation that is associated with carcinoma formation in the mouse breast.

### HISTOLOGIC CHANGES IN BREASTS OF ESTROGEN-TREATED FEMALES

*Paris Strain.*—The breasts of female mice of the Paris strain that have been treated with estrogen show intense stimulation. The whole organ is enlarged to several times its normal size, as shown in figures 12 *A* and *B*, of dissections of inguinal breasts from a normal mouse and a mouse treated for fourteen months, respectively.

Histologic study shows proliferation of all the glandular elements, which are closely packed together throughout the entire breast. Figure 13 *A* shows a representative section of a breast of an animal that died with mammary carcinoma after ten and two-tenths months of treatment. Ducts of all sizes are seen, usually surrounded by a number of budding acini. These acini proliferate to form broad fields, in which small solid

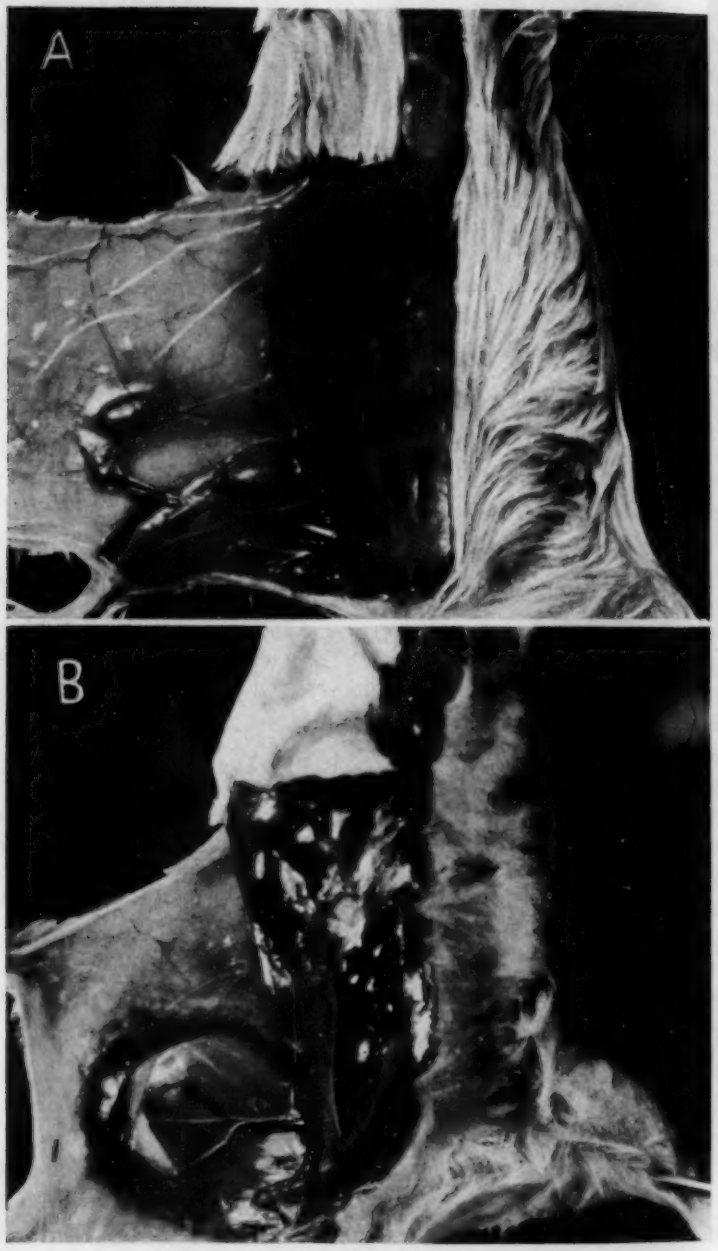


Fig. 12.—*A*, the gross appearance of the inguinal breast of an untreated female mouse of the Paris strain. *B*, the gross appearance of the inguinal breast of a female mouse of the Paris strain treated with estrogen.

glandular areas, representing early carcinoma, appear. The cells lining the acini often betray their malignant tendency by their increased size, hyperchromatism, mitosis and irregular arrangement two layers deep.

Fig. 13.—*A*, the breast of a female mouse of the Paris strain treated with estrogen. *B*, cystic dilatation of the ducts in the breast of a female mouse of the Paris strain treated with estrogen.

Cystic dilatation of the ducts is often seen in the breasts of these treated mice of the Paris strain and is one of the features which differentiates the breast of an estrogen-treated animal from that of a female

control mouse. Figure 13 *B* shows a breast in which this cystic change is well developed. It was from a mouse which had been treated for seven months and in which mammary carcinoma developed. It will be noted that there is no intraductal proliferation.

To sum up, we can say that estrogen treatment produces marked epithelial proliferation as well as cystic change in the breasts of Paris female mice. In this strain of mice the response of the female breast to estrogen appears histologically to be identical with the response of the male breast.

*Marsh Strain.*—The breasts of female mice of the Marsh strain treated with estrogen showed little if any evidence of growth stimulation of the epithelial elements. There was frequently dilatation of the ducts

TABLE 5.—*The Estrone-Treated Females*

| | Paris Strain | Marsh Strain | C57 Strain |
|---|---|---|---|
| Total group: mice surviving 4 mo..... | 111 | 85 | 85 |
| Percentage in which mammary carcinoma developed ..... | 74.77% ± 4.12% | 21.18% ± 4.43% | 0% ± 1.16% |
| Mean age in mo. at death..... | 7.82 ± 0.23 | 9.14 ± 0.32 | 12.07 ± 0.51 |
| s of age in mo. at death..... | 2.41 ± 0.16 | 2.95 ± 0.23 | 4.74 ± 0.36 |
| Median age in mo. at death..... | 7.60 ± 0.29 | 8.80 ± 0.40 | 12.30 ± 0.64 |
| Mice in which carcinoma developed..... | 83 | 18 | 0 |
| Mean age in mo. at death..... | 8.25 ± 0.24 | 10.25 ± 0.48 | |
| s of age in mo. at death..... | 2.14 ± 0.17 | 2.04 ± 0.34 | |
| Median age in mo. at death..... | 7.80 ± 0.30 | 9.90 ± 0.60 | |
| Mice in which carcinoma failed to develop ..... | 28 | 67 | 85 |
| Mean age in mo. at death..... | 6.54 ± 0.50 | 8.85 ± 0.38 | |
| s of age in mo. at death..... | 2.66 ± 0.36 | 3.09 ± 0.27 | |
| Median age in mo. at death..... | 6.10 ± 0.63 | 8.40 ± 0.48 | |

to a marked degree, but this was not accompanied by any intraductal proliferation. No increased budding of the ducts was produced by the treatment. When the breasts of these treated animals are compared with those of untreated bred controls, there is no apparent increase in the glandular elements. A typical breast from 1 of these treated Marsh females is shown in figure 14 *A*. This animal died with carcinoma after nine and four-tenths months of treatment.

We can conclude that although estrogen does cause cystic changes in the breasts of the Marsh females, just as it does in all other mice, it fails to stimulate the epithelial elements to proliferate.

*C57 Strain.*—In about three fourths of the female mice of the C57 strain treated with estrogen the breasts showed no effect other than cystic dilatation of the ducts. This sometimes reached an extreme degree as shown in figure 14 *B*. This mouse died after fourteen and three-tenths months of treatment.

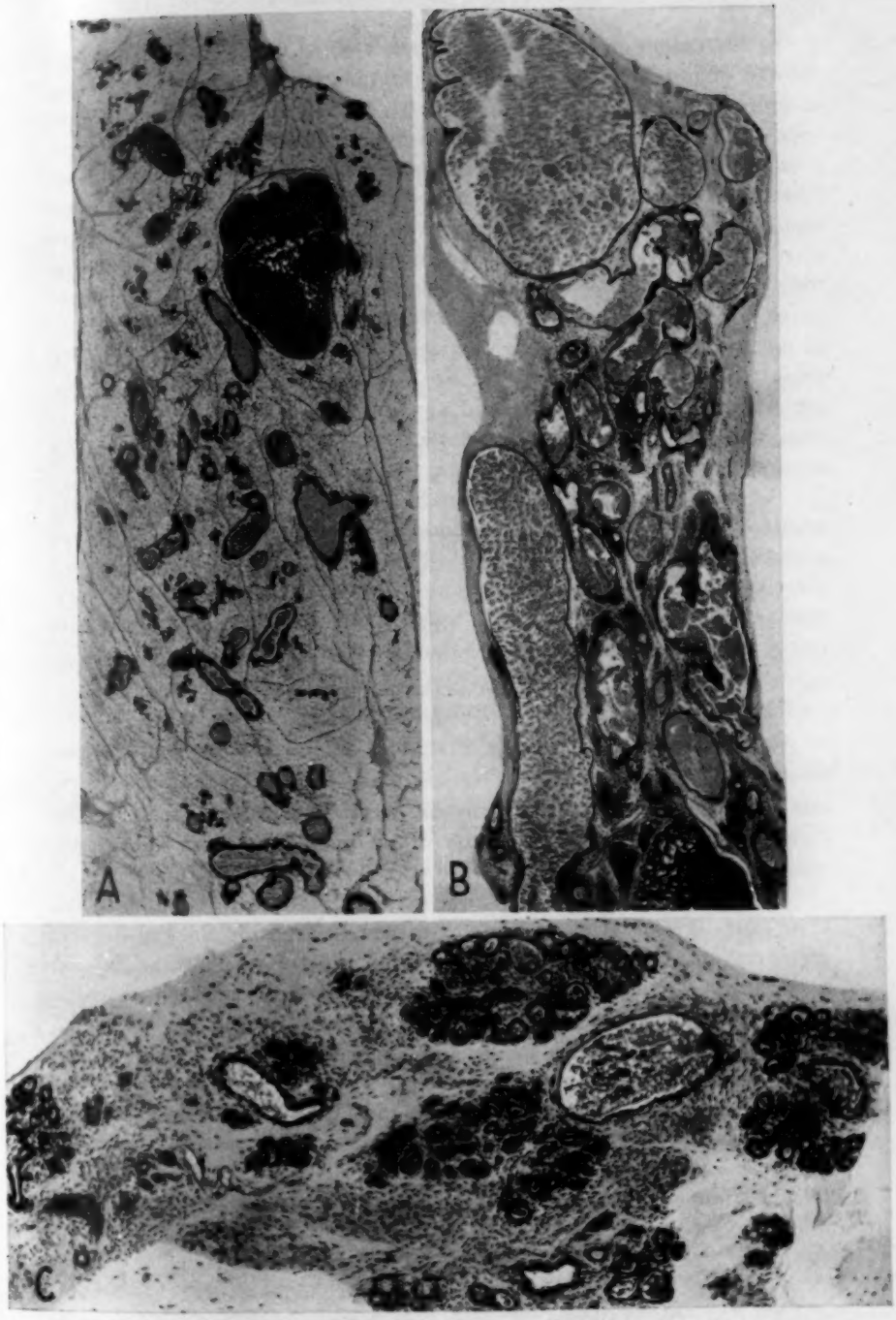


Fig. 14.—*A*, the breast of a female mouse of the Marsh strain treated with estrogen. *B*, the appearance of the breast in the majority of female mice of the C57 strain treated with estrogen. *C*, budding of acini seen in a minority of female mice of the C57 strain treated with estrogen.

In the remaining one fourth of these treated C57 females the breasts showed definite evidence of epithelial stimulation in the form of budding of acini from the ducts. Groups of from four or five to fifteen or twenty such acini were seen, as illustrated in figure 14 *C*. This breast was from a mouse that had fourteen and three-tenths months of treatment. Such extensive budding of the acini was never seen in the untreated control females of this strain. It is important to note, however, that although the estrogen induced a certain amount of budding of the ducts, the process did not go on to the formation of large fields and circumscribed solid masses of acini such as were regularly seen in breasts from other strains of mice in which carcinoma developed. Nor did estrogen produce in these C57 females the cytologic signs of malignant transformation, for the cells lining these budding acini were not enlarged and hyperchromatic, they did not show mitoses, and they maintained the arrangement of an orderly single layer that is usual in the ducts.

We can conclude that estrogen causes marked cystic change in the breast of the C57 females, but induces proliferation of the ducts in only a small proportion of the animals. This proliferation does not show all the characteristics of that which occurs in mouse breasts in which mammary carcinoma develops. The response of these female C57 mice to estrogen is, in general, similar to that in the males of the strain.

### EFFECT OF BREEDING ON INCIDENCE OF MAMMARY CARCINOMA

In the bred control mice of the Paris strain there was an increase in the incidence of mammary tumors over that in the unbred controls that was statistically highly significant. In the Marsh and C57 strains the incidence of tumors was also higher in the bred animals, but the increase was not statistically significant.

It is of interest to note that the tumor incidence was higher in the bred Paris mice even though the life span of the animals was shorter, for breeding shortens it. Both the mean and the median age showed a statistically highly significant shortening in the bred group as a whole, in the bred mice in which carcinoma developed and in the bred mice in which it failed to develop. In the Marsh strain the life span of the bred mice in which carcinoma developed was somewhat shorter, but the difference was not statistically significant. In the C57 strain there was also a slight shortening of the life span, enough to be suggestive of statistical significance.

The tumors developed at a considerably younger age in the bred animals. In the Paris strain this difference was statistically highly significant, while in the Marsh strain it was only suggestive.

Andervont[14] has written a good review of this question of the effect of breeding on the incidence of mammary carcinoma in mice. Although

14. Andervont, H. B., and McEleney, W. J.: Pub. Health Rep. **53**:777, 1938.

reports have conflicted, in general the disease has been found to occur more frequently and earlier in mice that have been bred. This was the case in the C3H strain with which Andervont himself worked.

It would seem that the evidence is fairly clear that breeding causes mammary cancer to develop more frequently and at an earlier age in some, though not in all, of the strains that have a marked predisposition to it.

### EFFECT OF ESTROGENS ON INCIDENCE OF MAMMARY CARCINOMA IN MALE MICE

The increase in the incidence of mammary tumors as the result of estrogen treatment in the Paris males—from 1.42 per cent to 87.50 per cent—is so great as to make statistical comparison superfluous when the number of animals is as large as it is in our data. In the Marsh males the increase was much less striking—from 0 per cent to 14.63 per cent—but it was still great enough to be statistically highly significant. In the C57 males the treatment had no effect. In all three strains the treatment caused a statistically highly significant shortening of the mean life span.

It has been suggested that all the estrogen does in these male mice is to produce in them a system of mammary glands that contain potentialities of malignant change similar to those inherent in the female mice of the same strain. Our data suggest that something more than this occurs, for the incidence of the tumors induced by the estrogen in the Paris males (in which it was highest) was statistically significantly higher than the incidence in the treated females of the same strain.

### DO ESTROGENS INCREASE THE INCIDENCE OF MAMMARY CARCINOMA IN FEMALE MICE?

It might be said that the most interesting point to be determined from these experimental data is whether or not estrogen treatment truly increases the incidence of mammary carcinoma in female mice. In the C57 strain no tumors at all developed in the treated females. In the Paris strain no statistically significant increase in the tumor incidence could be demonstrated in our data if the bred controls were used as a standard. If the incidence of tumors in the treated animals is compared with that in the unbred controls, however, it is at once seen that treatment causes a statistically significant increase. The mice treated with estrogen of course bore no litters, for the pathologic changes produced by the massive doses of estrogen that they received prevented pregnancy. Yet we cannot be sure that the factor that raises the incidence of mammary tumors when animals are bred did not also operate in these estrogen-treated females; the stimulation that the mammary gland

receives from pregnancy may be similar to that which it receives from injected estrogen. It would seem that all that we are justified in concluding is that estrogen administered in large doses to female mice failed to raise the tumor incidence beyond that produced by a normal physiologic process, i. e., pregnancy.

In the females of the Marsh strain treatment caused a drastic reduction in the incidence of tumors. Comparison of the life span in the control with that in the treated animals, however, at once shows that this factor played an important part in this change. The mean age at death of the treated Marsh females was 9.14 months, while it was 12.54 months in the bred controls and 14.06 months in the unbred controls—

TABLE 6.—*Expected Incidence of Mammary Carcinoma in Control Females at Median Age of Treated Mice in Which Carcinoma Developed* *

| | Median Age at Death of Treated Mice with Carcinoma, Mo. | Incidence of Carcinoma in Treated Mice | Expected Incidence of Carcinoma in Bred Controls | Expected Incidence of Carcinoma in Unbred Controls |
|---|---|---|---|---|
| Paris strain..... | 7.6 ± 0.3 | 74.77% ± 4.12% | 17.15% ± 3.70% | 4.26% ± 2.86% |
| Marsh strain..... | 9.9 ± 0.6 | 21.18% ± 4.43% | 41.75% ± 7.62% | 21.60% ± 5.76% |

* This calculation was made as follows:

1. Take given age (in this case the median age at death of the treated mice with mammary cancer).
2. Make this the new median age in the carcinoma group with which a comparison is desired.
3. Determine the number of mice below, and one-half the number of mice that fell in the month group thus selected, and add these two figures.
4. Multiply the resultant figure by 2.
5. Use this product divided by the total number of mice in the carcinoma group with which a comparison is desired as a fraction of the per cent of the original cancer incidence in the strain. This will give the expected incidence at the desired age.
6. Calculate standard errors of per cent.

differences that are highly significant statistically. Comparison of the life span in the treated with that in the control animals in the Paris and C57 strains likewise reveals that the estrogen caused a shortening of the life span that was statistically highly significant.

How important a role this shortening of the life span by the treatment really played in changing the incidence of tumor development can be determined by statistically predicting the expected incidence of carcinoma in the control mice at the median age at which tumors developed in the treated mice. This comparison is shown in table 6.

This comparison proves that the estrogen treatment brought on mammary carcinoma at a much earlier age in the Paris females than that at which such carcinoma usually develops in untreated bred or unbred mice. No such advancement of the age incidence of the disease occurred in the Marsh strain, however. These calculations also prove that the paradoxic lowering of the tumor incidence in Marsh females by the

estrogen treatment, which we have pointed out, was due merely to the reduction in the life span of the mice without a concurrent change in the age at which carcinoma developed.

### MAXIMAL AND MINIMAL DOSES OF ESTROGEN

The fundamental purpose of our experiment was to determine the effects on tumor incidence of the largest possible doses of estrogen. We did, however, treat a small series of mice with smaller doses. A total of 53 males and 51 females were given from four to six doses only of 0.05 cc. of estrone benzoate in oil suspension at weekly intervals. These mice were then allowed to live out their natural life. At death most of them still showed the evidences of estrogen effect even though their treatment had been limited to six weeks of injections begun when they were 2 weeks of age. The male mice treated in this way included 35 from the Marsh strain and 18 from the C57 strain. In none of them did carcinoma develop. It would appear, therefore, that this kind of treatment was not sufficient to induce tumors in males. The female mice that received this limited treatment presented tumors with about the same frequency as the untreated female controls of the same strain. The numbers of animals treated were so small, however, that we do not attach any statistical significance to these results.

### EFFECT OF ESTROGEN ON THE INCIDENCE OF TUMORS OTHER THAN MAMMARY CARCINOMA

It has been claimed by others that estrogen treatment induces in mice several types of cancer other than mammary carcinoma, including "spindle cell sarcoma," lymphosarcoma and epithelioma of the cervix. Lacassagne [3e] reported a thymic tumor and a cervical tumor diagnosed as epithelioma developing in estrogen-treated mice from a strain in which tumors did not develop spontaneously. Gardner and his associates [9b] reported the development of "spindle cell" sarcoma in all of 5 male mice from two different litters of the C3H strain that were given injections of estrogen for a long time. The tumors developed in the backs at the sites of injection, and the New Haven authors concluded that they had been induced by the estrogen. Gardner, Kirschbaum and Strong [9f] reported that the incidence of lymphoid tumors, leukemic and non-leukemic, was increased in a group of 149 mice of the C3H strain that were given injections of estrogen.

The capacity of estrogen to intensify in a striking manner the squamous character of the epithelium of the vagina and cervix is well known to all of those who have studied the effects of estrogens in mice. In many of the animals that receive a large amount of estrogen over a

long period the cervical epithelium is greatly thickened, and in some of these animals irregular strands and masses of cells appear to grow downward into the underlying connective tissue. Epithelial pearls develop in these masses, and the whole process has a superficial resemblance to early epithelioma, although it should be emphasized that irregularity in size and shape of the cells and mitoses are not seen to the extent that they occur in true epithelioma. Loeb and his associates did not regard these changes as fully developed epithelioma, preferring to call them "precancerous" and "carcinoma-like" processes. They found them in 10.7 per cent of a total of 234 mice receiving injections of estrogen. Gardner and co-workers,[9d] however, in 1938, described a large tumor of the cervix diagnosed as carcinoma, with metastases to the lum-

TABLE 7.—*Incidence of Lymphoblastoma and Other Nonmammary Forms* of Cancer*

| Strain | Type of Cancer | Control Mice | | | Treated Mice | | |
|---|---|---|---|---|---|---|---|
| | | Mice | Tumors | Per Cent Tumors | Mice | Tumors | Per Cent Tumors |
| Paris | Lymphoblastoma | 375 | 32 | 8.54 ± 1.45 | 331 | 5 | 1.51 ± 0.67 |
| | Other nonmammary types | | 1 | 0.27 ± 0.26 | | 0 | 0 ± 0.30 |
| Marsh | Lymphoblastoma | 180 | 4 | 2.22 ± 1.10 | 235 | 13 | 5.53 ± 1.49 |
| | Other nonmammary types | | 4 | 2.22 ± 1.10 | | 0 | 0 ± 0.42 |
| C57 | Lymphoblastoma | 289 | 10 | 3.46 ± 1.07 | 217 | 4 | 1.84 ± 0.91 |
| | Other nonmammary types | | 9 | 3.11 ± 1.02 | | 3 | 1.38 ± 0.79 |

*A wide variety of types of malignant tumors were found. They included carcinoma of the lung, leiomyosarcoma of the intestine, squamous cell epithelioma of the skin, rhabdomyosarcoma of the abdominal wall, liver cell carcinoma, carcinoma of the stomach, fibrosarcoma of the chest wall and osteogenic sarcoma of the femur.

bar lymph nodes, which developed in a C3H mouse given injections of estradiol benzoate for two hundred and sixty-six days. In 1939 Gardner and Allen [9e] reported finding a total of 27 "epithelial lesions" of the cervix or vaginal fornix among a total of 183 mice from eight different strains treated with estrogen. They classified three of these lesions as carcinoma and six others as "pre-malignant" lesions. Recently Allen and Gardner [9h] have described "lesions and carcinomas" of the cervix appearing in 56.8 per cent of 44 hybrid mice treated for a long time with estrogen.

Although the vagina, cervix and uterus were carefully inspected and sectioned in our mice we found no instance of squamous proliferation that we could classify as fully developed epithelioma. The examples of proliferation that we studied were sometimes striking, but they did not in our opinion show unequivocal characteristics of malignant change. They resembled very closely the squamous cell proliferation that is seen

in the epithelium of the human tongue overlying granular cell myoblastoma. This lesion has occasionally led pathologists to the mistaken diagnosis of squamous cell epithelioma of the tongue.

We cannot confirm from our data the induction of fibrosarcoma by injections of estrogen. Tumors of this type developed in only 2 of our total of 783 treated mice. These tumors both appeared in C57 mice. Neither of them arose in the region in which the injections were made, one developing in the axilla and the other in the lateral thoracic region.

Our experience as regards lymphoblastoma likewise shows nothing to indicate that the incidence of this type of tumor was increased by the injections of estrogen, as Gardner and his associates reported it to be. In table 7 we have recorded the incidence of lymphoblastoma (including both leukemia and lymphosarcoma) and of other nonmammary forms of cancer in our control and treated mice separately. Study of it will show that no statistically significant increase in the incidence of lymphoblastoma occurred in the treated mice.

### DOMINANCE OF HEREDITY OVER ESTROGEN EFFECT

There are several kinds of evidence from our experiment that suggest that the constitutional factors inherited by the mice of one of these so-called pure strains are dominant over the effects of estrogen treatment. Thus in the males the incidence of induced tumors roughly parallels the natural incidence of the disease in the females, and it also parallels the capacity of the estrogen to stimulate the breasts, as indicated by histologic study. The stimulation was slight in the breasts of the C57 mice and no tumors resulted, while it was intense in the breasts of the Paris mice where the incidence of induced tumors was high. Since the amounts of the estrogen administered to the mice of the different strains were similar, we should have expected a similar response if there were no intervention of some hereditary factor.

While the histologic effects of the treatment varied in the different strains, it was remarkably similar in the males and females of each strain. Cystic change was induced in a varying degree in all the mice, but the budding of ducts to form fields of acini such as are ordinarily associated with carcinoma development was not induced by the treatment except in mice from strains in which the phenomenon occurred naturally. This again suggests the dominance of a constitutional factor inherent in the strain.

The fact that the C57 strain mice had a considerably greater tolerance for the estrogen is another indication that some constitutional difference is involved.[15]

15. Little, C. C.: Am. J. Cancer **27**:551, 1936.

## SUMMARY AND CONCLUSIONS

The analysis of our data suggests that estrone benzoate in oil given to the limit of tolerance to mice of pure bred strains has the following results:

It increases the incidence of mammary carcinoma in the males of the high cancer strains, bringing up the incidence in the Paris R3 males to a level that is somewhat higher than that in female bred controls.

It fails to increase the frequency of mammary carcinoma in female mice of the high cancer strains above the natural frequency in bred female controls.

It lowers the age at which mammary carcinoma develops in the female mice of the Paris R3 high cancer strain.

It does not induce mammary carcinoma in either males or females of the low cancer C57 strain.

The histologic evidences of estrogen stimulation in the breasts of these strains of mice parallel the effects on tumor incidence.

The estrogen treatment did not induce sarcoma, lymphoblastoma, epithelioma of the cervix or any other form of extramammary cancer in the mice.

Hereditary factors are dominant over the effect of the administration of estrogen on the incidence of mammary carcinoma.

# THE HISTOGENESIS OF OVARIAN MESONEPHROMA

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In 1939, after having examined a great number of cystic and adenopapillary tumors of the ovary, I tried to establish some of them as belonging to a new group of ovarian tumors to which I gave the name "mesonephroma." This new type of cystic ovarian tumor has the following morphologic characteristics: the cells lining the cystic cavities are of an endothelial and not of an epithelial type as in the common type of cystoma, and the tumor tissue contains specific structural units, which duplicate the embryonic glomerulus. I suggested the following histogenetic hypothesis: The tumor develops from misplaced fetal remnants of the mesonephros. This abnormal inclusion and persistence of mesonephric tissue in the field of the ovary can occur easily, for the mesonephros develops adjacent to the hilus of the ovary. These two organs are secondarily separated only by two folds which develop between them, thus producing the ovarian ligament.

Since my first paper on this subject, I have had the opportunity to study 24 more tumors of this group. The assumed origin of mesonephroma by developmental error was previously supported in 2 cases which were reported in my first paper: case 9, that of a 21 year old patient with an ovarian teratoma presenting skin, sebaceous and sweat glands, teeth, bones and malignant mesonephromatous tissue and case 10, that of a 26 year old patient with malignant embryoma of the right ovary combined with dysgerminoma and mesonephroma. Dr. I. Davidsohn, Mount Sinai Hospital, Chicago, gave me the opportunity to examine slides of a similar case: that of a 12 year old girl with a football-sized dermoid of the left ovary which in addition contained typical mesonephromatous tissue. The tumor was removed three years ago, and there has been no recurrence.

All the tumors which I examined showed the characteristic lining of endothelial hobnail-shaped cells. Some of them, however, presented focal transition to cuboidal and even low columnar cells lining the characteristic capillary tufts of the glomerulus-like units. This finding can be easily explained by the mesonephric origin. The inner visceral

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Aided by a grant of the Scientific Committee of the American Medical Association.

glomerular leaf of Bowman's capsule develops as columnar epithelium in early fetal time and only later by flattening assumes its final endothelial or perithelial character.

The developmental changes in the visceral layer of Bowman's capsule in embryonic and early postnatal life have recently been studied by Gruenwald and Popper.[1] They found that even in early postnatal life the peaks of the loops are still covered with high columnar epithelium. Risak[2] described several cases of high cuboidal or even columnar epithelium in one or both leaves of Bowman's capsule in the kidney of the adult.[3] In mesonephroma this transition cannot be found in the cystic cavities, which in general are lined with typical hobnail-shaped or flat endothelial cells. By malignant proliferation, these cells may form solid masses of stellated cells, which are connected by thin projections, forming a loose cellular network with wide meshes. These solid parts of the tumors present the character of embryonal mesenchyma, corresponding to the mesodermal origin of the endothelial cells (fig. 1*A*). If present, the high cuboidal and/or the columnar cells of epithelial character are found only in two locations of the tumor, in the structure I called glomerulus-like, and in small adenomatous areas which duplicate neoplastic renal tubuli.[4] On the other hand, I have carefully examined slides of numerous specimens of serous and pseudomucinous ovarian cystoma. Ovarian cystoma develops from inclusions of the ovarian surface epithelium and duplicates by its prosoplastic potencies the prospective potencies of the müllerian duct. This epithelium has been incorrectly called the germinal epithelium. This name should be discarded, for it is known today that the germinal cells do not arise from the ovarian surface epithelium but originate from the epithelium of the hindgut. Serous cystoma duplicates tubal epithelium; endometrioma duplicates endometrium; pseudomucinous cystoma duplicates cervical mucosa. I have seen in serous cystoma inclusions of pseudomucinous cells and vice versa, but I have never seen endothelial cells in cystoma of any of the three mentioned types. The epithelium may become flattened by the pressure of the secretion but it still retains its epithelial character. Only the rare so-called microcystic or parvilocular type of cystoma of the ovary sometimes presents a lining of some of the small cystic cavities

1. Gruenwald, P., and Popper, H.: J. Urol. **43**:452, 1940.

2. Risak, E.: Virchows Arch. f. path. Anat. **267**:222, 1928.

3. Möllendorf, Borgmann and others compared the cells which cover the glomerular loops to pericytes; Clara, to the cells which cover the alveolar capillaries of the lung, the epicytes. Thus the nomenclature varies, but the origin of these cells is definitely mesodermal.

4. Schiller, W.: Am. J. Cancer **35**:1, 1939 [fig. 15].

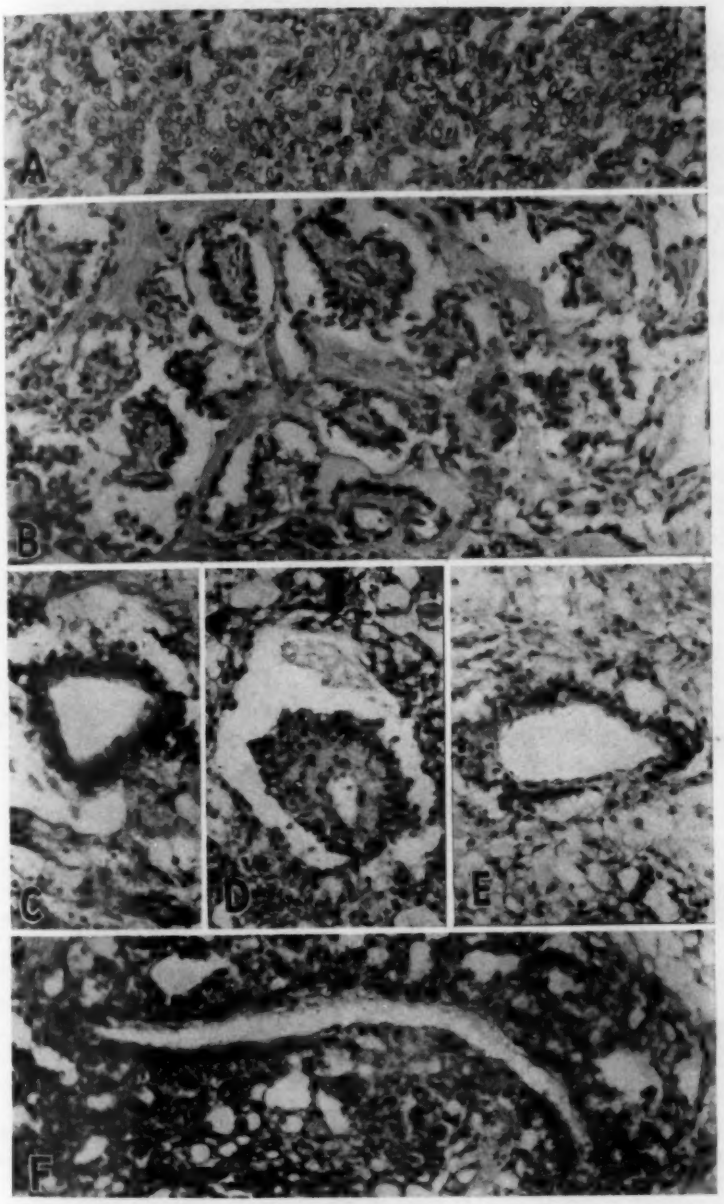


Fig. 1.—*A*, solid stroma formed by malignant proliferation of stellated endothelial mesodermal cells. × 130. *B* to *F*, glomerulus-like units of various tumors diagnosed as mesonephroma, *E* and *F* with progressive malignant proliferation of the lining of the capillary loop; in *F* the capillary is cut longitudinally. × 140.

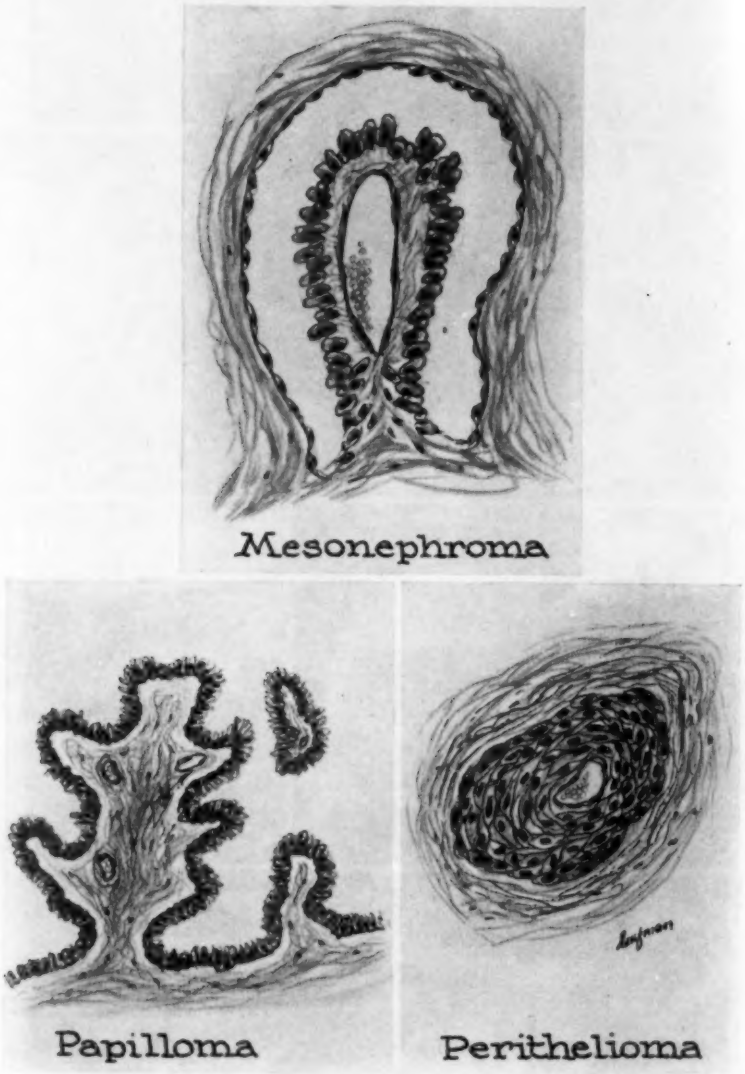


Figure 2

*(See legend on opposite page)*

which to a certain degree looks similar to endothelial cells. This similarity apparently is only morphologic, being due to the peculiar type of round cells with large nuclei and with a small amount of pale protoplasm, and is not histogenetic. Never did I find true endothelial cells in epithelial cystoma. Furthermore, if epithelial cystoma undergoes carcinomatous degeneration, it produces solid masses of cells of epithelial character, definitely different from the loose meshy cellular network in mesonephroma. Thus the conception of these tumors as representing an entity different from that of other cystic ovarian tumors seems to be well founded and established. It has been accepted by investigators who have published records of their own cases.[5]

The second part of my paper, the hypothesis of the mesonephric origin of the tumor, has met with certain doubts. Kazancigil, Laqueur and Ladewig [5c] published 3 of their own cases and tried a plastic reconstruction in their case 2, in which they failed to find any renal structure, a finding which I demonstrated by a similar reconstruction in 1 of the cases described in my paper. But the failure may be due to the choice of a field which contains no glomerulus-like structure and to the low magnification (fifty times instead of at least one hundred and fifty times). However, their cases 1, 2 and 3 present most typical glomeruloid units, as can be seen in their figures 8-11, 12, 15, 19, 23

5. (*a*) Jones, H. W., and Seegar, G. E.: Am. J. Obst. & Gynec. **39**:322, 1940. (*b*) Tuta, J. A., and Siebel, J. E.: Arch. Path. **31**:386, 1941. (*c*) Kazancigil, T. R.; Laqueur, W., and Ladewig, P.: Am. J. Cancer **40**:199, 1940. (*d*) Karsner, H. T., in Karsner, H. T., and Hooker, S. B.: Year Book of Pathology and Immunology, Chicago, The Year Book Publishers, Inc., 1940, p. 272.

EXPLANATION OF FIGURE 2

The specific glomerulus-like unit of mesonephroma compared with the structure of papilloma and with that of perithelioma.

Mesonephroma: A glomerulus-like structural unit is seen. A small cavity containing one relatively large papillary projection, which consists of *one* capillary loop embedded in a thin, eventually edematous coat of connective tissue. Note the papillomatous projections covered with columnar epithelium, which may get flatter and even endothelial like or may show malignant proliferation; also, the cavity lined with endothelial-like cells.

Papillomatous cystoma: The large cavity contains numerous small and large, mostly ramified papillomatous projections, each consisting of a strong core of connective tissue containing one or more relatively small blood vessels. The projections and the cavity are lined with the same type of columnar epithelium.

Perithelioma: One or more capillaries or small vessels are embedded in a concentric mass of proliferated perithelial cells. There is no cavity around the vessels and the coat. The perivascular lymph space, if present, is filled and obliterated by the proliferating perithelial cells.

and 24. Plastic reconstruction of any one of these units would have yielded the same result as in my case, the structure of a fetal glomerulus. But the tumor parenchyma as such reproduced under low power magnification cannot be expected to duplicate the architecture of the renal

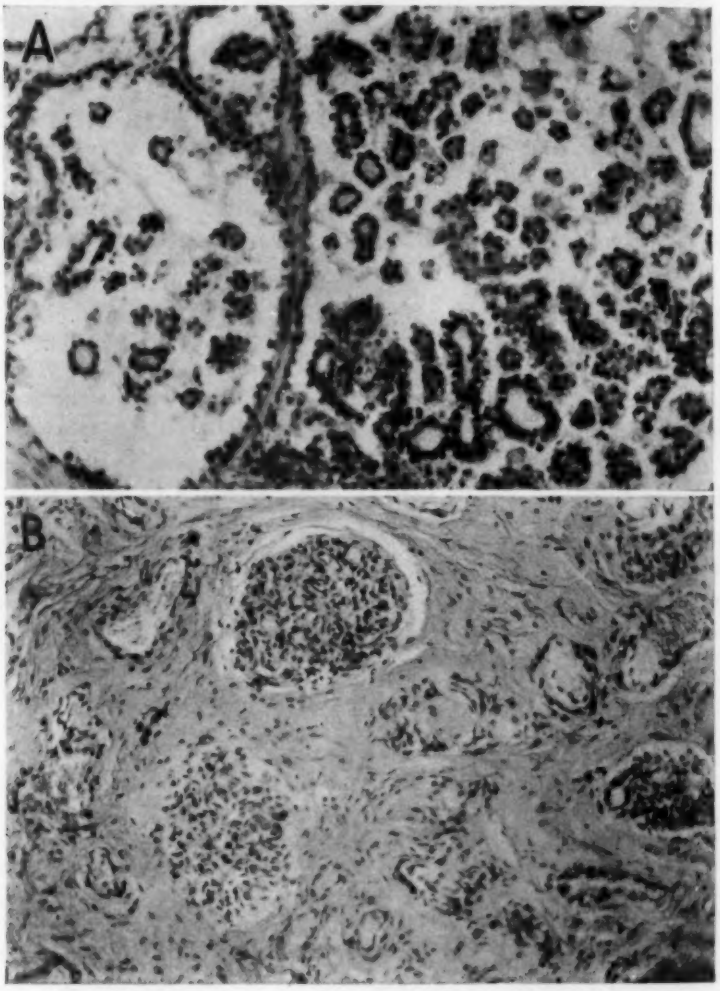


Fig. 3.—*A*, typical nonspecific papillary structure in a tumor diagnosed as mesonephroma: numerous small projections in relatively large cystic cavities. × 140. *B*, perithelioma of the skin of the scrotum: several capillaries embedded in a thick coat of perithelial cells. No distinct cavity can be seen around the capillaries. × 140.

cortex. Only the glomerulus-like units of the tumor correspond to the glomeruli of the fetal cortex. The authors cannot suggest a better

histogenetic explanation for the origin of the tumors in question. They satisfy themselves by suggesting the name "papilloendothelioma" founded on "the findings as a whole." This concept gives no adequate explanation for the specificity of the tumor in the ovary or for the specific perivascular structure, as they call it, or the glomerulus-like or glomeruloid structure, as I call it.

I still feel that this structure is a characteristic and typical one. It consists of a small cavity lined with endothelial cells which eventually become cuboid or low columnar. This structure never presents more than one papillomatous projection with one capillary loop in its center covered by endothelial or more frequently by cuboid or low columnar cells (fig. 2*A*). The similarity to a fetal glomerulus is striking. It can be overshadowed by neoplastic proliferation of one, in general the inner, or both linings, but the outlines of the structure, the small cavity with one capillary tuft, never change and are duplicated neither by the papillomatous projections in endothelioma or in epithelial cystoma nor by perithelioma. In epithelial papilloma the cavities are much larger and contain numerous papillary projections, most of them ramifying into smaller branches. In these projections the stroma is well developed and the vessels small. In mesonephroma there is one projection in a small cavity, with little stroma, which, in general, forms only a thin layer between the capillary wall and the covering endothelium. It never presents any ramification, nor is there ever more than one projection. In perithelioma the single vessel is surrounded by a dense coat of proliferated perithelial cells, which completely replaces the perivascular lymph space. The characteristic small cavity around the capillary tuft, which is never missing in mesonephroma, is not found in perithelioma, and the reconstruction of a tumor of that type shows longitudinally stretched vessels and never short loops or tufts (figs. 2 and 3). If the glomeruloid structure were an organ-unspecific perivascular mantle, developing from the perivascular lymph endothelium, we should expect to find the glomeruloid structure in tumors of all organs which contain vessels, in other words, in tumors of practically all organs. But it is found only in the endothelioma-like group of the ovarian tumors. This fits the conception of a developmental misplacing of mesonephric tissue into the field of the adjacent gonad, which may occur in either sex. Kazancigil, Laqueur and Ladewig discovered an analogous tumor of testicular origin in a man.[6] Through the courtesy of Dr. Tracy Mallory,

6. Whether the tumor in case 40 of A. R. Klossner (Arb. a. d. path. Inst. d. Univ. Helsingfors **10**:278, 1938), classified by Klossner as bidermoma or biphyllomatous teratoma, contained mesonephromatous parts cannot be decided by the description or the illustration. However, figure 96 is suggestive of glomerulus-

*(Footnote continued on next page)*

Boston, I received the slides and the history of a case which throws some new light on the origin of the glomeruloid structure and furnishes new evidence for its renal origin.

A 52 year old man four months prior to hospitalization had an attack of sudden pain in the right flank, without radiation but with soreness across the lower part of the abdomen. This pain was accompanied by nausea and vomiting and followed by hematuria a few days later. Two weeks before admission he had hematuria and on the day before admission he had a recurrence of the pain in the right flank, radiating to the right lower quadrant. Examination revealed a sense of fulness in the right loin, suggesting a large kidney.

The Hinton test was positive and the Wassermann test weakly positive. An intravenous pyelogram showed a filling defect in the right renal pelvis producing a deformity of the middle calix, measuring 3 cm. in diameter. The upper end of the ureter was dilated. The right kidney was removed, and the patient made an uneventful recovery.

The kidney measured 10 by 5.5 by 3 cm. and weighed 129 Gm. The capsule stripped with ease, revealing a smooth reddish surface, except for a slightly raised, dome-shaped yellowish red circumvallate area, measuring 2.5 by 3 cm., situated immediately lateral to the pelvis. The organ cut with normal resistance and showed slightly pale markings. The cortex measured 7 mm. in thickness. The pelvis appeared slightly dilated and contained two free soft pinkish yellow masses, 1.5 cm. in diameter. Attached to and growing from a calix near the lower pole of the kidney and opposite the previously described area on the external surface, there was a large soft reddish globular mass, measuring 2 cm. in diameter, which protruded into the pelvis.

The sections of the tumor presented an adenoma, which in some areas was cystic. The epithelium lining the ducts and cystic cavities duplicated more or less the renal tubular epithelium (fig. 4 *A* and *B*). The tumor showed in some areas typical glomeruloid structures, i. e., single tufts in small cavities, both lined with cuboidal epithelium, which represents a transition between immature fetal high columnar cells and mature flat endothelial cells (fig. 4 *C*, *D* and *E*).[7] Harmonizing with

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like structures. In case 41, called by Klossner an instance of triphyllomatous mixed tumor, figure 101 resembles the solid parts of mesonephroma. The combination of ovarian teratoma and mesonephroma was described in cases 9 and 10 in my first paper, and since then I have seen 1 more case. The combination of granulosa cell tumor and mesonephroma is represented by case 1 of Kazancigil, Laqueur and Ladewig.

7. In this instance renal adenoma is of a type different from the common cortical and eventually papillary adenoma, which frequently is encountered as multiple yellow subcapsular spots in sclerotic kidneys and probably gives origin to the so-called Grawitz tumors. These tumors originate from little cysts, which develop from terminal tubular loops walled off by the process of sclerotic shrinkage. It seems that the prosoplastic prospective potency which, if evoked, gives origin to adenopapillary hypernephroid tumors is widespread and rather common in the normal tubular epithelium. The cortical adenoma is of purely tubular origin and never shows glomerular-like structures. The tumor in question developed from the medulla, i. e., from a pyramid, evidently from some fetal remnant of metanephric tissue with tubular *and* glomerular potencies. Consequently, it produces adenoma of a tubular type *and* glomerulus-like structures.

the fact that the tumor clinically and histologically gave no evidence of malignancy, the epithelium consisted of only one regular row of cells and did not pile up toward Bowman's space. The only difference in comparison with the glomeruloid structures of ovarian mesonephroma was the slightly thicker wall of the vessel of the tuft as seen in some areas. This may have been due to the renal localization of the tumor as a result of secondary thickening. The presence of the glomeruloid structures in a tumor of evident renal origin supports the hypothesis of the mesonephric origin of the ovarian neoplasm which contains the same units.

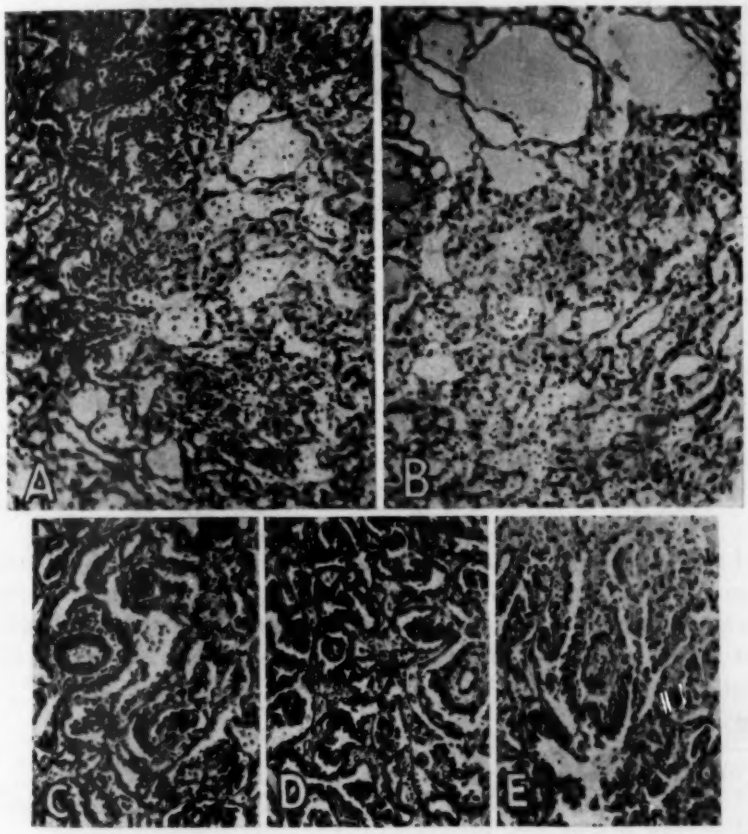


Fig. 4.—*A*, adenomatous and, *B*, cystic parts of a renal tumor; *C*, *D* and *E*, glomerulus-like units of the renal tumor. Note the similarity of the structure of the glomerulus-like unit on the right side of figure 4 *D* to the unit in figure 1 *D*. × 50.

SUMMARY

The mesonephric origin of the ovarian tumor called mesonephroma is supported by the study of a benign renal tumor from a 52 year old man. This tumor microscopically presented structures which were analogous to the glomeruloid units of ovarian mesonephroma.

1825 West Harrison Street.

# PRESENCE OF HISTAMINE IN INFLAMMATORY LESIONS

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Lewis and co-workers [1] suggested that a histamine-like substance is involved in the reactions of skin to injury. They expressed the belief that this substance, held normally within epidermal cells, is released by various types of injury and, diffusing out, causes the early changes of capillary dilation and permeability which in turn produce local edema and redness. Since this work, investigators have been intrigued by the idea that histamine may play a role in inflammation. That histamine is among the substances released by stimuli has been shown by Rosenthal and Minard.[2] Pursuing the ideas of Lewis [1] and Dale [3] that histamine is released by stimuli which change the permeability of the cell, one would be led to expect that the total histamine content of inflammatory areas would be decreased because of the diffusion of the released histamine. It has been found, however, that the opposite is true.

Loos [4] first showed that well developed inflammatory areas in rabbits contained amounts of histamine well above normal. Riesser [5] showed also that the lesions of the Arthus phenomenon were richer in histamine than the normal skin. Ellinger,[6] working on guinea pigs, and Tarras-Wahlberg [7] and Minard,[8] working on rabbits, found the same to be true of skin irradiated with ultraviolet rays.

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From the Research Laboratory, United States Marine Hospital.

1. Lewis, T.: The Blood Vessels of the Human Skin and Their Responses, London, Shaw & Sons, Ltd., 1927.

2. Rosenthal, S. R., and Minard, D.: J. Exper. Med. **70**:415, 1939.

3. Dale, H. H.: Lancet **1**:1179, 1929.

4. Loos, H. O.: Arch. f. Dermat. u. Syph. **164**:199, 1931.

5. Riesser, O.: Arch. f. exper. Path. u. Pharmakol. **187**:1, 1937.

6. Ellinger, F.: Arch. f. exper. Path. u. Pharmakol. **136**:129, 1928; **149**:343, 1930.

7. Tarras-Wahlberg, B.: Klin. Wchnschr. **16**:958, 1937.

8. Minard, D.: Studies on the Concentration and Liberation of Histamine in Tissues, Thesis, University of Chicago Graduate School, Chicago, University of Chicago Libraries, 1939.

It is clear that either the increased histamine is brought to areas of inflammation through the blood stream or else some normal local mechanism is stimulated to produce the increased amounts.

The findings of Loos [4] were explained by assuming that the leukocytes either formed or carried histamine. This was supported by work on animals in which the numbers of leukocytes had been decreased by benzene. Benzenized animals showed a poorer rise of histamine in inflammatory areas than control animals. Loos's point of view was supported further by the work of Code,[9] who also expressed the belief that white cells, particularly granulocytes, carried histamine.

More recent work [10] has shown, however, that in the rabbit the blood histamine is carried by the blood platelets and that the leukocytes carry little histamine, if any.

It is possible to remove the platelets in a highly specific manner by the use of antiplatelet serum. If the platelets are associated in any way with the inflammatory histamine, it should be possible to modify profoundly the histamine content of lesions by the use of antiplatelet serum.

The present experiments show that this expectation is fulfilled, and this finding furnishes evidence in favor of the idea that the increased histamine in inflammatory lesions is associated with the blood platelets.

## METHODS AND TECHNIC

The histamine equivalent was determined partly by the method of Barsoum and Gaddum [11] and partly by Code's [12] modification. With the rabbit under ether anesthesia, the skin was removed in amounts of 0.5 to 2 Gm. wet weight. This was placed in 10 cc. of 10 per cent trichloroacetic acid in a beaker. The tissue was minced with sharp scissors. The minced material remained in this first trichloroacetic acid extract twelve hours. This extraction was repeated for several hours. The moist tissue was washed on the filter with three successive portions of 2 cc. each of the trichloroacetic acid. After complete drainage, the tissue was transferred to tared watch glasses and dried at 90 C. to constant weight. It was found that about 10 per cent of the histamine activity was left in the tissue if only one extraction with trichloroacetic acid was used. The extracts were then refluxed ninety minutes with 10 cc. of concentrated hydrochloric acid, evaporated four times with 10 cc. of absolute alcohol under reduced pressure, extracted with distilled water, and neutralized with fifth-normal sodium hydroxide. In neutralizing, several drops of 0.05 per cent phenol red in distilled water was added directly to the water-extracted material, and base was added until the solution matched a standard at $p_H$ 7.4. The material was then titrated, the guinea pig ileum being used as the biologic test object.

In a number of determinations, the material was sent through an additional extraction of absolute alcohol as suggested by Barsoum and Gaddum.[11] This was

9. Code, C. F.: J. Physiol. **90**:485, 1937.

10. (*a*) Minard, D.: Am. J. Physiol. **119**:375, 1937. (*b*) Zon, L.; Ceder, E. T., and Crigler, C. W.: Pub. Health Rep. **54**:1978, 1939.

11. Barsoum, G. S., and Gaddum, J. H.: J. Physiol. **85**:1, 1935.

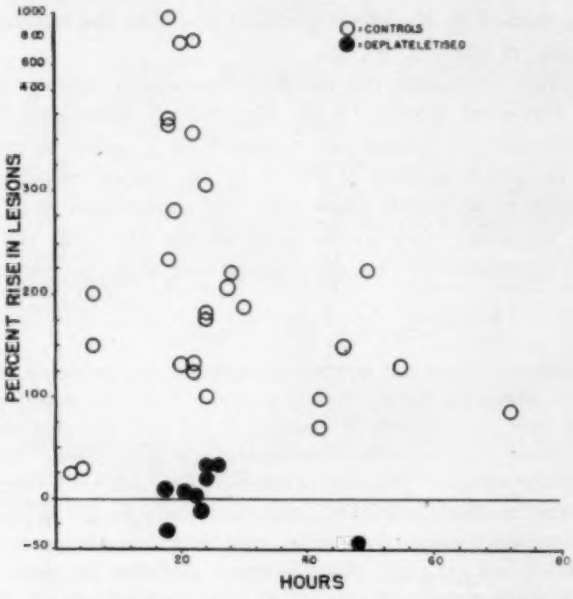
12. Code, C. F.: J. Physiol. **89**:257, 1937.

done to make certain that the fluctuations in histamine activity were not caused by the histamine-like alcohol-insoluble substance found by Anrep[13] to be present in red blood cells. These determinations on alcohol-extracted material gave lower absolute values than those on tissue not so treated. The ratio of lesion to control, however, was not materially affected.

Platelets were always counted by Tocantins' method.[14]

### THE HISTAMINE RISE IN INFLAMMATORY LESIONS

Rabbits previously sensitized intradermally to a killed vaccine of indifferent streptococcus were given intradermal inoculations of 0.3 cc.




The percental rise in histamine in streptococcic lesions in deplateletized animals and untreated controls.

of the vaccine. Within a few hours redness was visible, and within eighteen to thirty-six hours a firm red lesion between 1 and 3 cm. in diameter had formed. Andrewes,[15] Clawson [16] and others have described the immunologic features of such tuberculin-like allergic lesions. At various times after the inoculation, the lesions down to the fascial cleavage plane were removed along with a control piece of normal skin, generally

13. Anrep, G. V.; Barsoum, G. S.; Talaat, M., and Wieninger, E.: J. Physiol. **95**:476, 1939.

14. Tocantins, L. M.: Arch. Path. **23**:850, 1937.

15. Andrewes, C. H.; Derick, C., and Swift, H.: J. Exper. Med. **44**:35, 1926.

16. Clawson, B. J.: Arch. Path. **9**:1141, 1930.

situated laterally or above the lesion and occasionally on the opposite side from a symmetric spot. All the values were calculated in terms of micrograms of histamine base per gram of dry weight of tissue. The percental rise of histamine in the lesions as compared with the sample of normal skin was chosen as the means of comparison because the absolute values vary with the thickness of the hide of the individual rabbit. Results based on wet weight of tissue show less percental rise in histamine, probably because of edema fluid in the lesions.

The chart shows the results of these experiments. The peak of histamine concentration occurred at about twenty hours, although the lesions frequently did not reach maximum size until between twenty-four and thirty-six hours.

TABLE 1.—*Histamine Values (Micrograms per Gram of Dry Weight) for Turpentine Lesions on Normal and Deplateletized Animals*

| Rabbit | Control Skin | Lesion | Wheal | Platelets $\times 10^{-3}$ | Type of Lesion | Comment |
|---|---|---|---|---|---|---|
| 31 ..... | 3.26 | 3.70 | .... | ... | 0.3 cc. 5 hr. | Normal animal |
| 21 ..... | 6.75 | 10.00 | .... | 218 | 0.3 cc. 17 hr. | Normal animal |
| 44 ..... | 9.55 | 28.4 | .... | 489 | 0.3 cc. 18 hr. | Normal animal |
| 32 ..... | 4.33 | 8.2 | 20.0 | ... | 0.3 cc. 18 hr. | Normal animal |
| 44 ..... | 15.8 | 32.7 | 68.0 | ... | 0.3 cc. 24 hr. | Normal animal |
| 3 ..... | 7.1 | 17.2 | 25.2 | ... | 0.3 cc. 24 hr. | Normal animal |
| 55 ..... | 4.85 | 5.5 | 17.5 | ... | 0.3 cc. 24 hr. | Normal animal |
| 53 ..... | 26.4 | 77.0 | 64.0 | ... | 0.1 cc. 23 hr. | Normal animal |
| 70 ..... | 9.0 | 21.3 | 21.4 | ... | 0.1 cc. 22 hr. | Normal animal |
| 52 ..... | 4.7 | 13.2 | 14.7 | ... | 0.1 cc. 23 hr. | Normal animal |
| 33 ..... | 4.75 | 3.95 | .... | ... | 0.3 cc. 5 hr. | Deplateletized rabbit |
| 19 ..... | 5.9 | 1.44 | .... | 38 | 0.3 cc. 17 hr. | Deplateletized rabbit |
| 20 ..... | 4.0 | 2.84 | .... | 54 | 0.3 cc. 17 hr. | Deplateletized rabbit |
| 25 ..... | 12.0 | 8.80 | 8.30 | 45 | 0.3 cc. 24 hr. | Deplateletized rabbit |
| 43 ..... | 3.54 | 1.21 | 2.54 | 55 | 0.3 cc. 24 hr. | Deplateletized rabbit |
| 61 ..... | 5.10 | 4.15 | 3.93 | 74 | 0.1 cc. 22 hr. | Deplateletized rabbit |

When 0.1 to 0.3 cc. of turpentine is injected intracutaneously in the rabbit, within a few hours there is a large inflammatory wheal, 3 to 5 cm. in diameter, about a central white area of vasoconstriction. In table 1, under the heading "Wheal" is given the histamine content of skin samples taken from this wheal. The value given under "Lesion" is the histamine content of the central area. When there is no separate value given for the wheal, the value under "Lesion" includes the wheal, tissue having been taken including both.

Inflammatory lesions (not shown here) made with silica, aleuronat (an albuminoid substance and lecithin) and egg albumin in sensitized animals also showed an increase in histamine content.

The blood platelets were followed in several of the animals during the course of the lesions. The streptococcic lesions produced only slight change; the turpentine ones produced a fall followed by a rise in blood platelets.

Not every type of lesion shows a rise in histamine content. Minard [8] found a drop in burned areas on rabbits. The results of similar experience are shown in table 2. It may be noted that antiplatelet serum does not seem to affect the histamine content of burns.

### EFFECT OF ANTIPLATELET SERUM ON THE HISTAMINE CONTENT OF LESIONS

Rabbit platelet antiserum was made in guinea pigs by the technic of Bull and McKee.[17] Animals previously sensitized to streptococcic vaccine were treated with antiplatelet serum until their platelets had dropped to 30,000 to 100,000 per cubic millimeter. When the platelets were reduced, usually in about five hours, an intracutaneous injection of the same dose of vaccine as used previously was made.

TABLE 2.—*Effect of Burns on Histamine Content of Skin*

| Histamine in Normal Skin, Micrograms per Gram of Dry Weight | Histamine in Area of Burn, Micrograms per Gram of Dry Weight | Type of Lesion * | Treatment |
|---|---|---|---|
| 3.10 | 2.44 | 85 degrees 30 seconds 15 minutes | None |
| 4.25 | 1.6 | 85 degrees 30 seconds 24 hours | None |
| 2.72 | 1.55 | 85 degrees 30 seconds 24 hours | Antiplatelet serum |
| 8.7 | 7.2 | 85 degrees 30 seconds 42 hours | None |
| 8.55 | 7.95 | 65 degrees 30 seconds 3 hours | None |
| 10.7 | 9.2 | 65 degrees 30 seconds 3 hours | Antiplatelet serum |

* The temperature and the duration of the heat applied and also the age of the lesion are given.

From 0.1 to 0.3 cc. of turpentine was injected into deplateletized but not sensitized animals. The lesions were removed along with normal skin as previously.

It has been shown previously [10b] that when the platelets are reduced to between 100,000 and 30,000 per cubic millimeter there is a marked drop in blood histamine. In addition to platelet, white cell and red cell counts, done on every animal, a few histamine determinations were done during the course of lesions on deplateletized animals, and in each case these showed marked diminution, as expected. The blood picture in the deplateletized rabbits was similar to that presented elsewhere and showed a polymorphonuclear rise with a slight drop in lymphocytes.

The determinations on lesions of deplateletized animals are shown in the chart and in table 1.

The average increase in histamine in the streptococcic lesions was 19 micrograms per gram of dry weight, while the increase in deplateletized

17. Bull, C. G., and McKee, C. M.: Am. J. Hyg. **2**:208, 1922.

animals was 0.013 microgram. These figures are highly significant when tested by the statistical methods for small samples.

We have been able to prevent the rise in the histamine content of lesions by previous intravenous administration of saponin. This agent, however, as well as benzene, used by Loos,[4] has a profound effect not only on the platelets but on the erythrocytes and leukocytes as well. Intravenous administration of horse serum during the course of lesions has had no effect.

### THE HISTAMINE CONTENT OF LUNGS AND SKIN IN DEPLATELETIZED ANIMALS

The effect of antiplatelet serum on the histamine content of organs is of some interest. The lungs of 3 deplateletized animals contained much less histamine than the lungs of 3 normal controls. There were 12 normal animals from which skin samples had been assayed and from which at some later date skin was again assayed while the animal was deplateletized. These skin samples from deplateletized animals showed an average 35 per cent drop in histamine content as compared with the previous value for the same rabbit, and the mean values are statistically significant.

### EFFECTS OF ANTIPLATELET SERUM ON LESIONS FROM THE MORPHOLOGIC POINT OF VIEW

There has been no consistent correlation between the size of the lesion and the percentage rise in histamine. The more completely deplateletized animals have shown purpuric areas about the lesions, but the edema, turgor and redness of both streptococcic and turpentine lesions were not conspicuously different in the controls from these conditions in the deplateletized animals.

Microscopic study revealed that the number of polymorphonuclear cells present in the lesions was about the same in the deplateletized animals as in the controls. Because of difficulty in identifying platelets in tissue, studies by means of the usual platelet stains in tissue have been of no value.

In contrast with the effect of antiplatelet serum, the effect of saponin is marked. The effect of benzene has been discussed by Rich.[18] Lesions on animals in which the numbers of both platelets and white cells have been reduced by such an agent show striking decrease in size, turgor and degree of infiltration with polymorphonuclears.

### COMMENT

In order to maintain that the histamine increase in inflammation is caused by a local mechanism, one must assume that the diverse agents

18. Rich, A. R., and McKee, C. M.: Bull. Johns Hopkins Hosp. **54**:277, 1934.

antiplatelet serum, saponin and benzene all have the same direct effect on the enzymes producing histamine. Also it would be necessary to assume that the changes in blood histamine and blood platelets are merely incidental.

The simplest interpretation of the results is that platelets bring the increased quantity of histamine to the areas of inflammation.

Direct measurement gives a value of 0.4 cc. as the average volume increase in the streptococcic lesions. If we assume that the increase in the volume of the lesion is due solely to an increase in the blood content, we can account for an 0.8 microgram rise in histamine in the average lesion, since normal rabbit blood carries about 2 micrograms per cubic centimeter. The actual average rise was, however, 7 micrograms in the eighteen to twenty-six hour group. The direct measurement of volume errs heavily in a positive direction, as does the assumption that the total volume increase is due entirely to blood. It is difficult, therefore, to account for the histamine increase on the basis of the simple increase of blood content or extravasation of blood. There probably is some accumulation of platelets at the site of the lesion.

The experimental work on purpura has suggested that the platelets are in some association with the endothelium of blood vessels.[19] Danielli [20] has recently brought direct evidence to show that heparinized platelets may plug capillary defects and thus cut down passage of serum proteins and edema fluid into tissue spaces. The decrease in the histamine values of the normal skin and lungs of deplateletized animals as indicated in a foregoing paragraph also suggests that at least a portion of the normal histamine content of tissues is held in association with platelets which may be fulfilling some normal role in plugging capillary defects. Such a function might explain an increased number of platelets in the augmented capillary bed of inflammatory areas.

The lack of correlation between the histamine rise and the size and turgor of the lesion suggests that the histamine does not play the decisive role in the maintenance of these characteristics in the lesion. The activity of histamine depends, however, on other physiologic factors such as potassium ion concentration, $p_H$ and the presence of amino acids. The amounts of histamine remaining in the deplateletized lesions may conceivably be sufficient to produce physiologic effects.

These experiments, however, deal only with total content. They do not indicate how much of the histamine is held within tissue cells, how much is held by platelets within tissues and how much is free to perform physiologic functions. For these reasons, it is at the moment

U. S. Marine Hospital.

19. Bedson, S. P.: J. Path. & Bact. **24**:469, 1921.
20. Danielli, J. F.: J. Physiol. **98**:109, 1940.

impossible to speculate whether the histamine release in the early stages of inflammation is in any way related to the subsequent rise shown in these experiments.

## CONCLUSIONS

There is a significant rise of histamine content in various inflammatory lesions of the rabbit. This rise may be prevented by the administration of antiplatelet serum. The rise in histamine in inflammatory areas is possibly caused by the accumulation of platelets in the increased capillary bed of inflammatory areas.

# SERUM PHOSPHATASE ACTIVITY IN DISEASE OF THE LIVER

## CORRELATION OF THE SERUM ENZYME ACTIVITY AND THE HEPATIC HISTOLOGIC CHANGES

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This is the report of a study of alkaline serum phosphatase activity in hepatic disease. A close parallel was found between the degree of injury to hepatic cells and the level of the enzyme activity. It was also observed that severe damage of hepatic cells in various types of hepatic disease would markedly increase the serum phosphatase activity without necessarily producing jaundice. It was also revealed that hepatic metastases from carcinoma did not always cause demonstrable intrahepatic biliary obstruction. The data indicated that the use of the serum phosphatase determination as an aid in differentiating obstructive from nonobstructive jaundice is not satisfactory.

Roberts [1] first advocated the determination of the alkaline serum phosphatase as an aid in differentiating obstructive from nonobstructive jaundice. He believed that high values for serum phosphatase activity in cases of jaundice indicated an obstructive type of jaundice. Since then, other investigators [2] have supported this contention. Meranze, Meranze and Rothman [3] further noted that hepatic metastases from carcinoma give high values for serum enzyme in a high percentage of instances. Gutman and co-workers [2b] corroborated this observation.

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1. Roberts, W. M.: Brit. M. J. **1**:734, 1933.

2. (*a*) Flood, C. A.; Gutman, E. B., and Gutman, A. B.: Arch. Int. Med. **59**:981, 1937. (*b*) Gutman, A. B.; Olson, K. B.; Gutman, E. B., and Flood, C. A.: J. Clin. Investigation **19**:129, 1940. (*c*) Meranze, T.; Meranze, D. R., and Rothman, M. M.: Rev. Gastroenterol. **6**:254, 1939. (*d*) Winkelman, L., and Schiffmann, A.: Arch. Int. Med. **64**:348, 1939. (*e*) Snell, A. M., and Magath, T. B.: J. A. M. A. **110**:167, 1938. (*f*) Herbert, F. K.: Brit. J. Exper. Path. **16**:365, 1935. (*g*) Armstrong, A. R.; King, E. J., and Harris, R. I.: Canad. M. A. J. **31**:14, 1934.

3. Meranze, D. R.; Meranze, T., and Rothman, M. M.: Pennsylvania M. J. **41**:1160, 1938.

Both groups believed that these cases also belonged in the category of those of obstructive jaundice since, they contended, the carcinoma masses in the liver produced intrahepatic biliary obstruction. However, both groups noted that in a small number of cases with extensive metastases it was not possible to demonstrate any marked increase of the serum enzyme. In opposition to the majority of investigators, Bodansky and Jaffe,[4] Cantarow and Nelson [5] and Green, Shattuck and Kaplowitz [6] concluded from their investigations that the determination of the enzyme activity was of little or no practical value in differentiating the two types of jaundice.

## MATERIAL

The material consisted of 46 cases in which one or more of the following diagnoses were made clinically: jaundice, hepatomegaly, cirrhosis of the liver and carcinoma with or without hepatic metastases. Necropsy afforded a check on these diagnoses in 41 instances. In 5 cases of cirrhosis the final diagnosis was confirmed from biopsy material obtained at operation. Particular attention was paid to the microscopic appearance of the parenchymal liver cells.

The method of Bodansky [7] was employed in the determination of the alkaline serum phosphatase. Our values for normal adults of 1.5 to 4.0 units corresponded closely to those obtained by others [8] employing the same method. In addition, the icteric index was estimated in many cases, using a standard laboratory procedure.

For purposes of clarity, the terms employed for the recording of the histologic observations of the liver cells are defined. "Dissociation" means the fragmentation of the hepatic cell cords, referred to by Kirshbaum and Popper [9] as serous hepatitis. "Necrobiosis" means the early cellular degeneration manifested by impaired staining, poor delineation and bile pigment retention of the cells. Since normal hepatic cells extrude bile pigment,[9] the presence of this substance is believed to indicate necrosis or necrobiosis of the hepatic cell. The terms "amyloidosis," "fatty metamorphosis" and "necrosis" are self explanatory. No attention was paid to the cellular change known as parenchymatous degeneration since it may be an agonal or a postmortem phenomenon.

The 46 cases were divided into three groups. Group I consisted of 27 cases which clinically presented either hepatomegaly alone or jaundice with or without hepatomegaly. In 3 cases there was in addition metastatic skeletal carcinoma of a type known not to affect the serum phosphatase to any marked degree. In group II were 9 cases of hepatic cirrhosis. No distinction was made between the Laennec and biliary types. Group III consisted of 10 cases of extrahepatic cancer without evidence of hepatic disease. Clinically these cases presented neither hepatomegaly nor jaundice, and at autopsy both liver and skeleton were free of metastases. These cases served as controls and afforded a basis of comparison for the hepatic histologic studies.

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4. Bodansky, A., and Jaffe, H. L.: Proc. Soc. Exper. Biol. & Med. **31**:107, 1933.

5. Cantarow, A., and Nelson, J.: Arch. Int. Med. **59**:1045, 1937.

6. Green, C. H.; Shattuck, H. F., and Kaplowitz, L.: J. Clin. Investigation **13**:1079, 1934.

7. Bodansky, A.: J. Biol. Chem. **101**:93, 1933.

8. Gutman and others.[2b] Bodansky.[7]

9. Kirshbaum, J. D., and Popper, H.: Arch. Int. Med. **65**:465, 1940.

GROUP I. HEPATOMEGALY; JAUNDICE

Group I was subdivided into two parts, the first consisting of 18 cases with serum phosphatase activity above 10 units, and the second, of 9 cases with this activity below 10 units. This arbitrary figure of 10 units was chosen since Gutman and associates [2b] considered this point the dividing line between obstructive and nonobstructive jaundice. In 6 of the 18 cases (1 to 6) there was complete extrahepatic biliary obstruction by carcinoma. In 9 cases (7 to 15) there was carcinoma with metastases to the liver. The latter cases are similar to those which Meranze and co-workers [3] and Gutman and co-workers [2b] considered instances of intrahepatic biliary obstruction. In 3 cases (16 to 18) there was neither extrahepatic nor intrahepatic biliary obstruction nor carcinoma. Case 16 was that of a patient with chronic alcoholism whose liver showed severe fatty metamorphosis; case 17 was that of a patient with marked amyloidosis of the liver, and case 18, that of a patient with pernicious anemia and marked passive engorgement of the liver.

A marked similarity in range of serum phosphatase activity was noted in the first 18 cases. In the 6 cases with extrahepatic biliary obstruction, the values for the serum enzyme ranged from 10.4 to 31.9 units. In the 9 cases with hepatic metastases from carcinoma or with so-called intrahepatic biliary obstruction, the values ranged from 11.4 to 36.9 units. In the last 3 cases the values ranged from 11.2 to 30.7 units.

The icteric indexes varied markedly in contrast to the values for serum enzyme activity. In the cases with extrahepatic biliary obstruction, the values were high, varying from 91 to 350. In the cases with hepatic metastases from carcinoma the values were decidedly lower, ranging from 5 to 90, and in 1 instance (case 13) the value was normal. The patient with chronic alcoholism, whose serum phosphatase value was 13.0 units, had an icterus index of 90. The patient with amyloidosis had a serum phosphatase value of 30.7 units and a normal icterus index of 6. The patient with pernicious anemia and a serum phosphatase value of 11.2 units had an icterus index of 10.

A gradual fall in the serum enzyme from a high level to a lower or a normal level was noted in 3 cases (6, 10, 15) although further progress of the disease was noted in each instance. Case 6, in which there was carcinoma of the gallbladder with complete extrahepatic biliary obstruction and several hepatic metastases, revealed a fall in the serum phosphatase values from 10.4 to 3.0 units. In case 10, with hepatic metastases from carcinoma, the phosphatase values rose from 8.1 to 18.1 units and then fell to 14.4 units. In case 15, also with hepatic metastases from carcinoma, the phosphatase values fell from 14.6 to 7.5 units. There was a marked concomitant rise in the icteric indexes in cases 6 and 15, in the

former from 65 to 350 and in the latter from 56 to 77. There was little or no change of the icterus index in case 10.

The common finding in the histologic study of the liver in the entire group of 18 cases was the extreme damage to the hepatic cells. In the cases with extrahepatic biliary obstruction there was extensive necrosis of the cells in the central zones of the liver lobules. When the added factor of hepatic metastases was present, there were extensive zones of necrobiosis and necrosis of the parenchymal cells surrounding the malignant masses. The same change was noted about the carcinoma masses in the 9 cases with hepatic metastases only. In the latter cases, most striking was the necrosis of the central cells of the liver lobules in areas free of metastases. However, in only 2 of these cases was there histologic evidence of intrahepatic biliary obstruction. In these the small duct radicles and canaliculi were distended and filled with bile. The liver of the patient with chronic alcoholism showed intense fatty metamorphosis as well as foci of necrosis of the central liver cells. The liver of the patient with amyloidosis revealed marked pressure atrophy of the hepatic cell cords. That of the patient with pernicious anemia showed severe passive congestion, which had produced widespread central cell necrosis.

Serum phosphatase values below 10 units were present in the last 9 cases (19 to 27) of group I. In none of these was there extrahepatic biliary obstruction. In 5 cases (19 to 23) there were hepatic metastases from carcinoma. The size and number of the latter ranged from a few small nodules to many of massive size. In 1 instance (case 20) the serum phosphatase activity, which had been normal, became slightly elevated following laparotomy.

The icteric indexes ranged from 3 to 135 in 4 of the 9 cases. The highest was obtained in case 19, an instance of carcinoma of the gallbladder and cystic duct. Among those cases in which the icteric indexes were not determined, clinical jaundice appeared following laparotomy in only 1 instance (case 20) and was absent in the remaining 4 cases.

Extensive damage of liver cells was noted only in case 19. The changes were similar to those noted in the 18 cases already discussed. In addition there was histologic evidence of acute suppurative cholangitis and intrahepatic biliary obstruction. However, there were only seven small hepatic metastases. The changes noted in the remaining 8 cases were in sharp contrast to those found in the cases already discussed. Slight or moderate necrobiosis or necrosis of the central cells and of the cells surrounding the carcinoma masses was found in 4 cases (20 to 23) with hepatic metastases. Moderate central necrobiosis and passive engorgement of the liver were present in 2 cardiac cases (25 and 26). Moderate central necrobiosis and dissociation of the liver cell cords were noted in 2 cases, case 24, with septicemia, and case 27, with uremia.

### GROUP II. HEPATIC CIRRHOSIS

A serum phosphatase value above 10 units was present in 1 case of cirrhosis (case 36). The initial value in this case was 13.0 units. It then dropped to 9.5 units, subsequently rose to 14.0 units and finally fell to a normal level of 3.6 units. In the other 8 cases the values ranged from 4.3 to 8.4 units.

Jaundice was present in 7 of the 9 cases. In these the icteric indexes ranged from 24 to 210. In 4 instances jaundice was clinically suggestive of intermittent biliary obstruction.

Extensive degenerative changes of the parenchymal cells was present only in the single instance in which the serum phosphatase activity was markedly elevated (case 36). Here, large areas of necrosis of the hepatic cells were noted in the central as well as in the peripheral zones of the cirrhotic nodules. In the other 8 cases, varying degrees of necrosis and fatty metamorphosis affected chiefly the peripheral cells of the nodules. For the most part, however, the hepatic cells were either of normal or of hyperplastic appearance.

### GROUP III. EXTRAHEPATIC CANCER AS CONTROL

The serum phosphatase values in the 10 control cases were below 5 units in 9 instances and 5.9 units in 1 (case 42). The icteric indexes ranged from 3 to 7 in 4 cases. In the 6 cases in which the icteric indexes were not determined, jaundice was not noted clinically.

The hepatic cells were normal or showed minimal degenerative changes in 8 of the 10 cases. Marked fatty metamorphosis of the hepatic cells was present in case 42, with a serum phosphatase value of 5.9 units. Marked dissociation of the liver cell cords was noted in case 39. In the latter a serum phosphatase value of 2.6 units was obtained prior to laparotomy, and the patient died two weeks later of acute diffuse peritonitis.

### COMMENT

Our data agreed in several respects with those of other investigators. Thus it was noted that extrahepatic biliary obstruction was usually associated with high values for serum phosphatase activity. The material also confirmed the observations of Meranze and co-workers [3] and Gutman and associates [2b] that hepatic metastases from carcinoma do not always markedly increase the activity of the serum enzyme. Further, only a single case in this series was similar to those reported by Franseen and co-workers [10] and Gutman and associates,[2b] in which marked increases in serum phosphatase activity were present in patients with hepatic metastases from carcinoma who were free of jaundice.

10. Franseen, C. C.; Simmons, C. C., and McLean, R.: Surg., Gynec. & Obst. **68**:1038, 1939.

The theory advanced by Gutman and associates [2b] to explain the increase of serum phosphatase activity in disease of the liver fails to account for some observations in this study. Gutman and collaborators expressed the belief that a "retention" of the enzyme occurs in the presence of biliary obstruction. One would therefore expect that a definite parallel would exist between the degree of icterus and the serum phosphatase activity. Such a parallel was not observed. Actually a marked difference was noted in the degree of jaundice in the cases of intrahepatic and extrahepatic biliary obstruction, while the serum phosphatase values for both types of cases were similar. Furthermore, the theory would scarcely explain those few cases of obstructive jaundice in which a gradual fall was noted in the serum phosphatase values, while jaundice became more intense. The theory also fails to explain the several cases without jaundice or with nonobstructive jaundice in which the serum phosphatase values were markedly elevated. The cases referred to are, specifically, those of the patient with amyloidosis, the patient with chronic alcoholism whose hepatic cells showed severe fatty metamorphosis and the patient with pernicious anemia who had marked passive engorgement of the liver.

The contention advanced by Meranze and associates [3] and Gutman and co-workers [2b] that hepatic metastases from carcinoma produce intrahepatic biliary obstruction is also not entirely confirmed by our material. In only 3 of 14 cases of this type was there positive histologic evidence of intrahepatic biliary obstruction.

Another theory is suggested by our observations. Since it was found that the degree of damage to hepatic cells closely paralleled the elevation of the serum phosphatase activity in the majority of cases, it appears reasonable to conclude that they stand in causal relationship. It also appears reasonable to believe that the damaged cells release the active enzyme into the blood stream. The degree of injury to hepatic cells then would be the determining factor governing the level reached by the enzyme. It also is evident that the damage to the parenchymal cells may be caused by a variety of conditions, not all necessarily associated with biliary obstruction.

Recent experimental observations seem to lend support to the theory advanced. By special staining methods, Gomori [11] demonstrated that the enzyme tends to be concentrated in the cells of the central zone, the cells of the peripheral zone or the cells of both zones of the liver lobule. Takamatsu [12] observed that injured liver cells give a more strongly positive reaction for the enzyme than do normal hepatic cells. Kabat and Furth [13] with similar experimental work were somewhat less specific

11. Gomori, G.: J. Cell. & Comp. Physiol. **17**:71, 1941.
12. Takamatsu, H.: Tr. Jap. Path. Soc. **29**:492, 1939.
13. Kabat, E. A., and Furth, J.: Am. J. Path. **17**:303, 1941.

in reference to the parenchymal hepatic cells. Kabat,[14] however, succeeded in liberating the active enzyme into suspension fluid by producing autolysis of the enzyme-bearing cells in vitro. Thus, it is conceivable that necrosis of the hepatic cell is the in vivo parallel process. The central hepatic cells, which are also enzyme-bearing cells, are the cells which, as has been noted, early undergo necrobiosis and necrosis in a variety of diseases of the liver. Therefore, any disease process which produces necrobiosis and necrosis of these hepatic cells may in so doing liberate the enzyme from the cells, thus increasing the enzyme in the blood serum.

The results obtained in 4 cases in this series appear to contradict the explanation just advanced. These were the cases of extrahepatic biliary obstruction, hepatic metastases from carcinoma, and cirrhosis in which the serum phosphatase values gradually fell to low or normal levels despite evident progress of the disease. However, a hypothesis is advanced which can reconcile this apparent discrepancy. There probably is a stage in hepatic cell necrosis when the liver cell becomes completely depleted of the enzyme. At this point even complete dissolution of the hepatic cells would not increase the amount of enzyme liberated into the blood serum. However, since staining methods for the demonstration of the enzyme in tissue were not carried out in this study, no definite proof can be advanced for this hypothesis.

## SUMMARY

A study of 46 cases in which alkaline serum phosphatase activity was estimated is reported. They included cases of jaundice, hepatomegaly, cirrhosis, carcinoma with or without hepatic metastases and in several instances miscellaneous liver diseases. In 3 cases there was metastatic skeletal carcinoma of a type known not to affect markedly the serum phosphatase activity. Necropsy was done in 41 cases; biopsy of the liver, in 5 cases. Particular attention was paid to the microscopic alterations of the hepatic cell. The analysis indicated that the serum phosphatase activity in the absence of skeletal disease depends initially at least on the degree of damage to hepatic cells. It also appears that injury to hepatic cells is produced by a variety of diseases of the liver with or without biliary obstruction and is not always associated with jaundice. The material further reveals that hepatic metastases from carcinoma do not necessarily imply intrahepatic biliary obstruction and finally that the determination of the serum phosphatase activity is not a reliable aid in differentiating obstructive from nonobstructive jaundice.

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14. Kabat, E. A.: Science **93**:43, 1941.

# LESIONS OF THE PURKINJE NETWORK OF THE BOVINE HEART AS A RESULT OF POTASSIUM DEFICIENCY*

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Sykes and Alfredson [1] described changes in the electrocardiograms of calves maintained on rations with a low potassium content. The Purkinje network of the heart of each of these calves has been examined, and the present paper concerns the changes observed under these conditions.

Sections from the hearts of 5 of 6 calves maintained on rations containing 0.10 to 0.12 per cent of potassium were obtained when the animals were killed. Sections from the hearts of 3 calves maintained on similar rations to which sufficient potassium was added to meet their requirements were likewise obtained. The tissues were fixed in a 4 per cent solution of formaldehyde, and the sections were stained with eosin-alum-hematoxylin.

Of the 6 calves on the deficient ration, 4 showed electrocardiographic changes. The other 2 calves in this group (C 410 and C 424) failed to show these changes, possibly owing to the fact that the serum potassium levels in these calves did not show the same marked drop as was noted in the other 4 calves. In fact, C 424 showed no change in serum potassium at any time. The type of electrocardiographic alterations observed are shown in figure 1.

Degenerative changes in the fibers of the Purkinje network were found in all animals which showed changes of any degree in their electrocardiograms, and in spite of the fact that calf C 410 showed no electrocardiographic abnormalities lesions were found in the Purkinje network. In the latter instance the changes were less marked and not so extensive as those in the other calves. Calf C 424 was finally dis-

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* Journal Article No. 552 (n. s.) from the Michigan Agricultural Experiment Station.

1. Sykes, J. F., and Alfredson, B. V.: Proc. Soc. Exper. Biol. & Med. 43:575, 1940.

carded, and sections of cardiac tissue were not available. No lesions were observed in the Purkinje network of any of the control animals.

The presence of the degeneration of the Purkinje fiber was denoted mostly by a granular appearance of the central portion of the cell as shown in figure 2. The granules in sections stained with eosin-alum-hematoxylin were a brownish yellow and reminded one of deposits of blood or of lipochrome pigment. The particles could be easily taken for red cells except for their location and variation in size. In some instances nearly the whole diameter of the cell showed the granular appearance, while in others only a few centrally located granules were noted. The condition resembled somewhat that described as brown

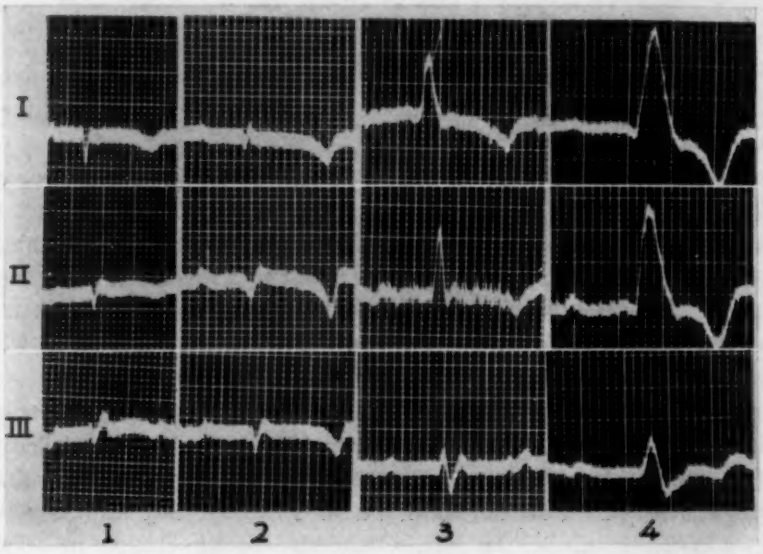


Fig. 1.—Electrocardiograms showing changes typical for the experimental calves: (1) control tracing; (2) tracing taken at 460 days of age; (3) electrocardiogram taken at 492 days; (4) electrocardiogram taken at 750 days of age. (From Proc. Soc. Exper. Biol. & Med. **43**:575, 1940.)

atrophy of the muscle fibers of the heart. The particles sometimes showed considerable size, as illustrated in figure 1, but this was not the general rule. It was not determined whether the larger particles were agglomerates of smaller ones or whether they developed as such from the start. In some slides the cells had a vacuolated appearance, as shown in figure 3, although this was not a common observation. The amount of change varied considerably from slide to slide from the same animal.

In this series of animals none showed deposition of calcium salts in the fiber system as previously observed in calves on rations deficient

in magnesium (Moore, Hallman and Sholl [2]). So far as could be determined, the myocardial fibers showed no alteration.

Aside from the problem presented here, a rather interesting observation was made in that a sarcocyst was encountered in a Purkinje fiber.

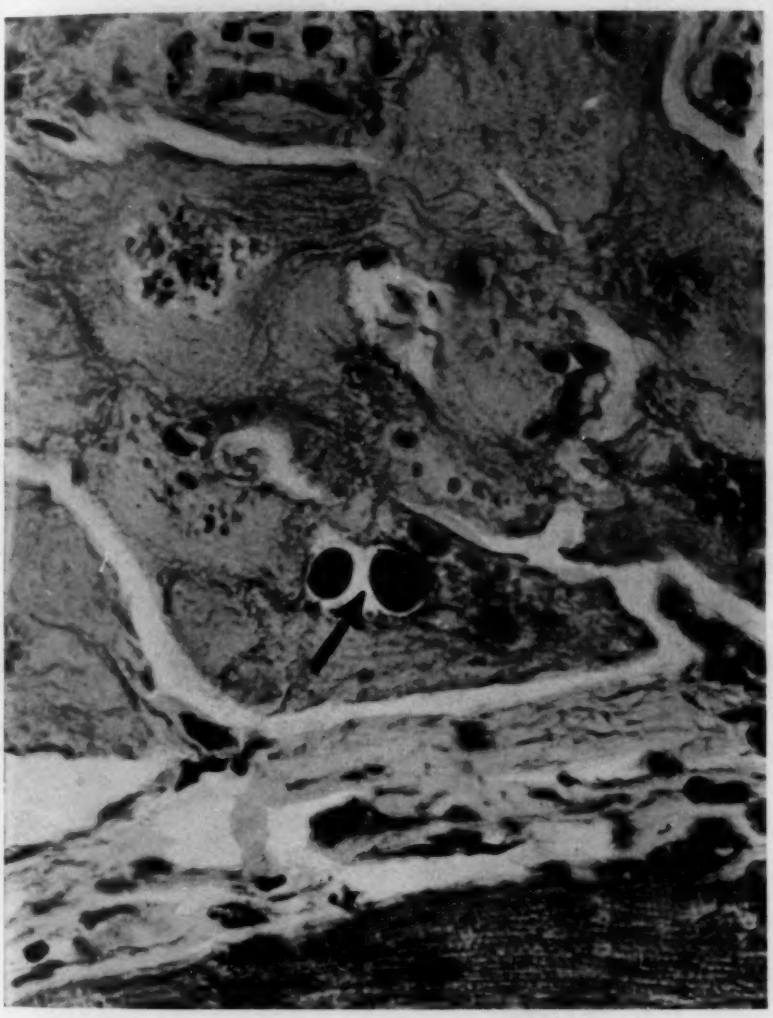

Fig. 2.—Degeneration of the Purkinje fibers. The arrow indicates the large granulations referred to in the text. Eosin-alum-hematoxylin stain; × 780.

While sarcocysts are often seen in bovine myocardial tissues it was thought worth while noting their presence elsewhere. This further emphasizes the close natures of the two types of cells.

2. Moore, L. A.; Hallman, E. T., and Sholl, L. B.: Arch. Path. **26**:820, 1938.

The changes noted in our animals were not in any respect similar to the changes noted by Thomas, Mylon and Winternitz [3] in rats and pigs on deficient diets. These investigators observed changes in the myocardial fibers which they ascribed to combined deficiencies of

Fig. 3.—The vacuolated appearance of the cells as observed in some sections is shown. Eosin-alum-hematoxylin stain; × 780.

potassium and vitamin B. We have never found evidence of myocardial damage in any of our animals, and the type of damage found in the Purkinje fibers was not at all comparable to that found in the myo-

3. Thomas, R. M.; Mylon, E., and Winternitz, M. C.: Yale J. Biol. & Med. **12**:345, 1940.

cardium by Thomas and his associates. Our animals also failed to show the marked symptom complex noted by these workers. In fact, they appeared normal at all times, no deaths occurred and changes in the Purkinje network as indicated by the electrocardiograms did not occur until the animals had received the deficient ration for several months. The discrepancy between these two sets of observations suggests that the myocardial lesions noted in rats and pigs were the result of vitamin B deprivation aggravated by potassium deficiency, and it is possible that if the animals had survived for longer periods the potassium deficiency would have produced lesions of the Purkinje fibers also, although the possibility of species differences cannot be ignored.

As noted, lesions quite similar to those observed in the present study have been described by Moore, Hallman and Sholl [2] in calves suffering from magnesium deficiency. Some calcification of the fibers was also observed in their calves. This calcification was not observed in the potassium-deficient calves. Similarly, the gross calcifications observed in the aorta and the ventricles and on the diaphragm of the magnesium-deficient calves were never in evidence in the potassium-deficient animals.

The similarity of the lesions in these two deficiencies is somewhat confusing. The potassium-deficient animals showed some slight variations in the magnesium content of the blood from time to time but never any change approaching the low levels observed in the magnesium-deficient calves. Similar small variations in blood magnesium were noted in the controls for the potassium-deficient animals and, as noted, no lesions were observed. Obviously, these slight variations in blood magnesium were not responsible for the lesions in the potassium-deficient calves. It likewise seems improbable that the lesions observed in the magnesium-deficient calves were due to potassium deficiency, since these animals were maintained almost exclusively on a whole milk ration which contains an abundance of potassium. One is forced to the conclusion that either potassium or magnesium deficiency will produce similar lesions in the fibers of the Purkinje network. In addition, rather widespread calcification seems to be characteristic of magnesium deficiency.

## SUMMARY

It has been shown that rations low in potassium when fed to calves produced degenerative changes in the Purkinje fibers of the heart. These changes were accompanied by alterations in the electrocardiograms of the animals. The Purkinje fibers presented in varying degree a granular appearance. Vacuolation of the fibers was also observed in some instances.

# A SINGLE STAGE METHOD TO PRODUCE BRAIN ABSCESS IN CATS

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In order to study in detail the histogenesis of brain abscess, or to investigate new technics of surgical treatment or of chemotherapy, there is need for a method of producing brain abscesses in laboratory animals. A number of procedures have been suggested, all of which have involved a preliminary operation to traumatize the brain followed after an interval by the inoculation of infected material. Malinowsky [1] reported the production of abscesses in dogs by puncturing the brain with a hypodermic needle and, after ten days, injecting a virulent culture into the punctured area. Essick,[2] using cats, injected broth cultures or dilutions of pus into previously damaged areas of brain and obtained acute abscesses which were often complicated by generalized meningitis. Groff [3] presented a series of five abscesses produced in cats by the injection of infected blood agar into the brain several days after the dura and cortex had been exposed and traumatized. The organisms used were Streptococcus pyogenes and Pneumococcus type III. He was unable to produce abscesses with Staphylococcus aureus or Streptococcus haemolyticus. Pilcher [4] found that abscesses sometimes occurred in dogs after the placing of contaminated foreign bodies in the brain. Markley [5] has recently shown that abscesses in the brains of dogs will result from the injection of a culture of Pneumococcus type III into cerebral tissue previously damaged by sodium ricinoleate.

In this paper, a method will be described which possesses the following advantages: It is a one stage procedure, consisting of a single injection through a small punch hole in the skull without exposure of the dura or artificial trauma of the brain. It can be completed in several minutes, and is suitable for injections made in a large series of animals. It is not limited to one organism; in this laboratory it has been used

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1. Malinowsky, V.: Centralbl. f. d. med. Wissensch. **29**:162, 1891.
2. Essick, C. R.: Arch. Neurol. & Psychiat. **1**:673, 1919.
3. Groff, R. A.: Arch. Neurol. & Psychiat. **31**:199, 1934.
4. Pilcher, C.: Ann. Surg. **103**:173, 1936.
5. Markley, G. M.: Proc. Soc. Exper. Biol. & Med. **47**:171, 1941.

successfully with the hemolytic streptococcus, Staph. aureus, Pneumococcus type III and Pneumococcus type XXII. The method is amenable to bacteriologic study under controlled conditions. Abscesses of varying chronicity may be produced, which may be studied at any stage in their development. The percentage of positive results is high enough to be satisfactory for experimental purposes.

The procedure has been employed during the past year in a study of the pathologic appearances and the experimental therapy of brain abscess, a study which is still in progress. This paper will be confined to a description of the technic and a brief presentation of the general results obtained.

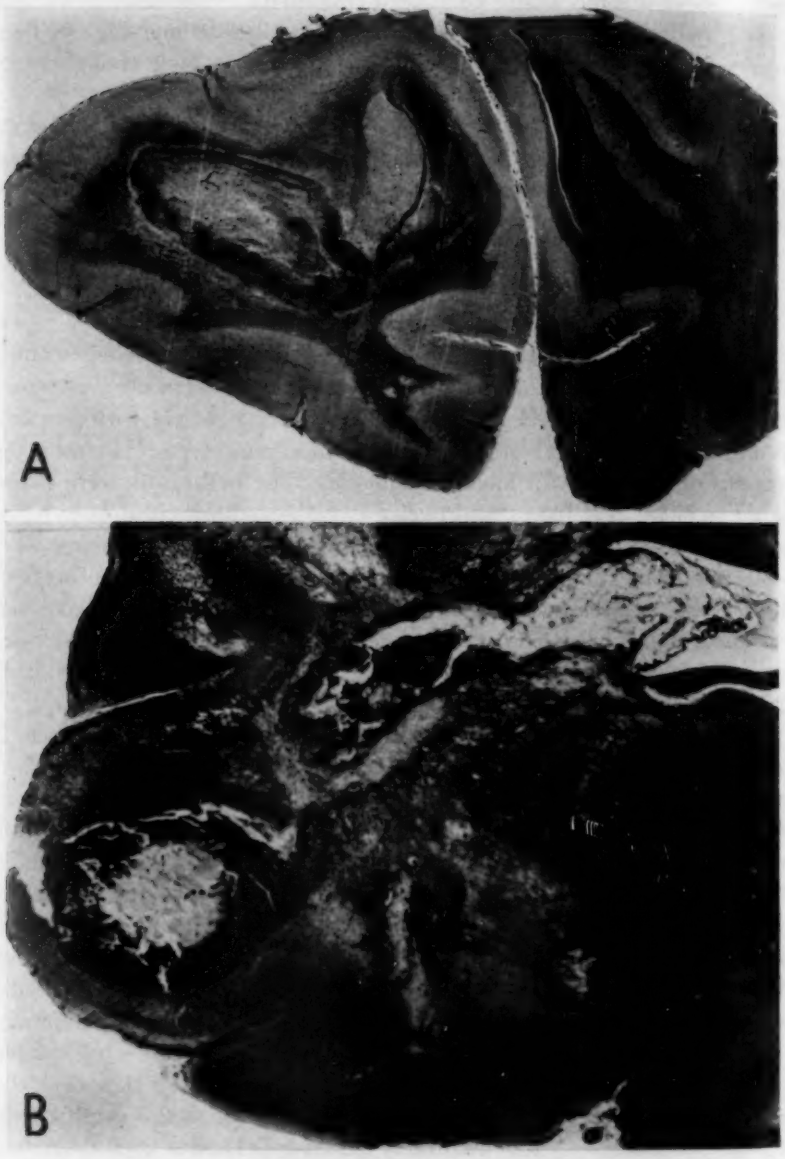
## METHOD

The following strains of bacteria were selected from cultures representing human infections: Staph. aureus from an infected wound, Str. haemolyticus from the blood culture of a patient with septicemia, Pneumococcus type XXII from the spinal fluid of a patient with purulent meningitis, and several strains of Pneumococcus type III from the sputums of patients with lobar pneumonia. The organisms were maintained on blood agar plates, and twelve to eighteen hour cultures in blood broth were used for inoculation. Whenever possible, the organisms were recovered from the experimental abscesses and used for subsequent inoculations. Special studies of the virulence of organisms have not been made, but it has been noted that certain organisms produce abscesses with almost unfailing regularity, while others yield less dependable results.

When a thin fluid such as broth is injected into brain substance, it does not become well localized and in many cases can be seen to flow back along the needle to the surface of the brain. Therefore, in order to obtain true localization of bacteria within the brain, material for injection was prepared as follows: A 2 cc. amount of the broth culture was mixed with an equal amount of melted agar which had cooled to approximately 45 C., and some of this mixture was immediately drawn into a tuberculin syringe and allowed to cool. This material, which had the consistency of jelly, could readily be injected through a 26 gage hypodermic needle and formed a small round pellet, sharply localized at the site of injection.

Adult cats were anesthetized by intraperitoneal injection of soluble pentobarbital U. S. P. A short incision was made in the skin over the right midparietal region and a small hole made through the skull with a pointed instrument. A tool admirably suited for this has been described by Syverton and Berry.[6] Through this hole a 26 gage hypodermic needle, attached to the loaded tuberculin syringe, was inserted for a depth of about 1 cm.; 0.05 cc. of the infected broth-agar mixture was then injected. During withdrawal from the site of injection, an electro-

6. Syverton, J. T., and Berry, G. P.: Science **86**:567, 1937.




*A* (cat 70), twenty-one day abscess caused by Staph. aureus. *B* (cat 60), eight day abscess with perforation into lateral ventrical, caused by Pneumococcus type XXII.

coagulating current was applied to the butt of the needle for about a second, in order to prevent contamination of the pathway of the needle through the surface of the brain. The cutaneous incision was closed with skin clips after removal of the needle. In some instances the electrocoagulating current was applied to the needle before the injection of organisms in order to create a local zone of necrosis in the brain, but this step was not found to be essential for the development of an abscess.

Four control animals were also inoculated in this manner with a sterile broth-agar mixture. These animals remained entirely well, and were killed between one and two weeks after receiving the injection. Their brains showed no evidence of the injected material on gross examination of brain sections, nor any destruction of tissue at the injection sites.

*Results of Intracerebral Inoculation of Eight Cats with a Strain of Staphylococcus Aureus*

| Cat | Course | Duration | Result |
|---|---|---|---|
| 63 | Apathetic and weak; coma and death on fourth day... | 4 days | Abscess |
| 65 | Remained in coma after operation; died after 36 hr. | 36 hr. | Acute necrosis, with bacteria in tissues |
| 66 | No symptoms or signs; killed after two weeks..... | | No abscess |
| 69 | Apathetic and weak; moribund on fourth day..... | 4 days (killed) | Abscess |
| 70 | Apparently well; killed after three weeks..... | 21 days (killed) | Abscess |
| 71 | Apathetic on 7th day; convulsions on 8th day..... | 8 days (killed) | Abscess |
| 75 | Apathetic on 6th day; coma and death on 7th day..... | 7 days | Abscess |
| 76 | Apparently well until 8th day, then comatose..... | 8 days (killed) | Abscess |

In several animals a different procedure was carried out, the infected broth-agar mixture being used in an attempt to produce experimental thrombophlebitis of the pial veins. Here the method consisted of removing a plug of bone with a small trephine over the parietal area, incising the dura, exposing the pia and injecting a small quantity of the material into the lumen of a pial vein. This injection was made through a 27 gage hypodermic needle, and produced immediate occlusion of the vein. The dura was then sutured, the bone replaced and the cutaneous incision closed. Section of the brain of one of these animals, killed after six days, showed an area of necrosis and inflammation in the occipital lobe, just posterior to the site of injection. There was also extensive thrombophlebitis of the pial veins with large masses of cocci contained in the capillaries and venules of the cortex. Further pathologic studies are to be reported at a later date, but the method is included in this report as a suggested means for the production of cerebral thrombophlebitis in cats.

## RESULTS

The results of the intracerebral inoculation in a series of 8 animals, with the same strain of Staph. aureus, are indicated in the table. Abscesses occurred in all but 1 of these animals. The age of the abscesses varied considerably, and the factors involved in this variation are a subject for further investigation.

Pneumococcus type III has been used in a large number of cats in this laboratory, with satisfactory results. In a recent series of 25 animals, abscesses were produced by this method in 17. An abscess has also been produced in 1 animal with the hemolytic streptococcus, and in 1 animal with Pneumococcus type XXII.

There appeared to be no criteria by which brain abscess could be diagnosed from the general appearance or behavior of the animals. Extensive abscesses were found in some which seemed to be quite well, while others became apathetic and refused to eat but showed no apparent neurologic abnormalities. A few cats became comatose, with rigid extension of the back and occasional clonic movements of all the limbs. One animal remained well until the tenth day and then died suddenly from rupture of an abscess into the lateral ventricle.

Detailed histologic studies will not be included in this paper. The general characteristics of the abscesses are illustrated in the figure. The microscopic appearance resembled that of early brain abscess in man, with a central necrotic zone containing many bacteria, surrounded by a wall of polymorphonuclear leukocytes, capillaries and compound granule cells, beyond which was a region of parenchymal degeneration and diffuse perivascular infiltration by lymphocytes. The older abscesses showed fibroblastic proliferation to varying degrees and deposition of connective tissue fibers. Some of the older lesions had undergone recent acute extension into neighboring tissue, with new abscess formation, while some had perforated into the lumen of the lateral ventricle. The leptomeninges showed mild infiltration by inflammatory cells in the vicinity of the abscess in most of the specimens, but diffuse purulent leptomeningitis did not occur.

## SUMMARY

A method is described for the production of brain abscesses in cats, which consists of a single stage procedure and which is believed to be satisfactory for experimental investigations into the histogenesis, course, bacterial factors and therapy of the brain abscess.

A variation of the method which can be used for the production of experimental thrombophlebitis in the pial veins of the cat is also described.

# ANACHORETIC EFFECT IN PULPITIS

## II. HISTOLOGIC STUDIES

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AND
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ST. LOUIS

The death of the dental pulp after slight injuries to the tooth or following operative procedures that are not usually considered injurious to the pulp has interested dental investigators for many years. The usual explanation given is that the peculiar circulatory system of the pulp cannot react to inflammatory changes in the usual manner and that the dilation of arteries in hyperemia must of necessity cause the collapse of the veins at the apical foramens, leading to thrombosis and "self strangulation." [1] We attempted to produce this condition experimentally in the teeth of cats and dogs but were unable to obtain the desired result in healthy animals. A rational review of the physical principles involved and an in vitro experiment [2] indicated that other causes should be sought. The work of Csernyei [3] on the role of anachoresis in periodontal conditions led us to try the effect of induced bacteremia in conjunction with irritation of the pulp. The bacteriologic aspects of the investigation, together with a discussion of the related literature, were described in an earlier paper.[4]

### EXPERIMENTAL PROCEDURE

Cats with fully erupted permanent dentitions were used as experimental animals. Canine, carnassial, second upper and second and third lower premolar teeth were chosen for experimental use because of their size. With the cat under

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1. Cahn, L.: Dent. Items Interest **48**:1, 1926. Hill, T. J.: J. Am. Dent. A. **21**:820, 1934. Kronfeld, R.: J. Dent. Research **15**:429, 1936; Histopathology of the Teeth, Philadelphia, Lea & Febiger, 1933, p. 106. Hopewell-Smith, A.: Dent. Cosmos **66**:489, 1924. Bunting, R. W., and Hill, T. J.: Oral Pathology, ed. 2, Philadelphia, Lea & Febiger, 1940, p. 240.

2. Boling, L. R., and Robinson, H. B. G.: J. Dent. Research **17**:310, 1938.

3. Csernyei, J.: J. Dent. Research **18**:527, 1939.

4. Robinson, H. B. G., and Boling, L. R.: J. Am. Dent. A. **28**:268, 1941.

anesthesia from intraperitoneally administered soluble pentobarbital U. S. P., cavities were cut as deep as possible in the dentin without exposing the pulp. Croton oil was immediately placed in the cavities to increase the irritation. From a few minutes to twenty days after the preparation of the cavity a suspension of streptococci or of staphylococci was injected into a vein of a hindleg. The operative and the bacterial technic are described at length in a previous publication.[4]

The cats were allowed to live for periods of from four to sixty days after the injection of the bacteria. At death the jaws were removed, roentgenographed and cut into blocks containing the teeth. The same tooth of the opposite jaw was used as a control. Ten parts of formaldehyde solution U. S. P. in 90 parts of physiologic solution of sodium chloride was used as a fixative for one week followed by decalcification in 5 per cent aqueous nitric acid. Sections were made by the celloidin or the paraffin method.

Eighteen cats with evidence of naturally acquired infections were also studied. This group included 1 cat with a large postaural abscess, 1 with ulcerated gingiva, 5 with infected eyes, 3 with fractured canine teeth showing evidence of periapical disturbance and 8 with pulps exposed at the time of cavity preparation. It was found necessary to include the latter as natural infections because the results of treatment were not at all comparable to the results in the other cats which received injections of bacteria but were comparable to those of the naturally infected animals. The histologic preparation was the same as that used with the animals receiving bacterial injections.

## OBSERVATIONS

*Pathologic Changes of the Pulp.*—The reactions of the pulp to the croton oil treatment of cavities appeared the same histologically whether the cat had been given an injection of a suspension of streptococci or staphylococci or had been naturally infected. The lesions can therefore be considered as a group instead of separately according to type of infection. Of the 83 experimental teeth studied, 90.4 per cent presented some form of visible reaction. These reactions could be divided into five types ranging in severity from hyperemia to complete necrosis of the pulp. The distribution of reactions is shown in table 1.

(*a*) Hyperemia. Simple hyperemia was found in less than 5 per cent of the experimental teeth examined. The vascular channels were dilated throughout the pulp, especially in the region of the cavity. Occasional small petechiae were found in the odontoblastic layer.

(*b*) Cellular infiltration. Teeth with cellular infiltration also showed some hyperemia. Leukocytes, mostly neutrophils (fig. 1 *A*), appeared in the more localized involvements near the site of the inflammatory agent. Numerous polymorphonuclear cells appeared in the blood vessels and in some cases could be found throughout the pulp.

(*c*) Pulpal abscesses. These were the most frequently found lesions in teeth of cats which had received injections of staphylococci or which had natural infections. The tissue at the center of an abscess, which in

some cases occupied over half of the pulp chamber, was completely liquefied. Usually only one center was found, but multiple abscesses were sometimes found. The remaining pulp tissue might appear normal or present hyperemia and infiltration (figs. 1 *B* and 2 *A*).

(*d*) Delimited necrosis. Reactions classed under this heading had the classic appearance of an anemic infarct but without evidence of thromboses. Karyorrhexis and karyolysis of the cells of the entire coronal and part of the root pulp were accompanied by dissolution of the connective tissue. The necrotic area was bordered by a dense infiltration of polymorphonuclear leukocytes and the adjacent blood vessels engorged with red cells (fig. 1 *B*). When seen in double-rooted

TABLE 1.—*Pulpal and Periodontal Response in Teeth of Cats Following Intravenous Injection of Bacteria or Associated with Naturally Acquired Infections*

| | Injection of Streptococci | | Injection of Staphylococci | | Natural Infection | | Total | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| Animals with croton oil treatment | 13 | .... | 5 | .... | 18 | .... | 36 | .... |
| Teeth treated with croton oil | 22 | .... | 11 | .... | 40 | .... | 83 | .... |
| Treated teeth with normal pulps | 3 | 9.4 | 0 | 0 | 5 | 12.5 | 8 | 9.6 |
| Treated teeth with hyperemia | 1 | 3.1 | 1 | 9.1 | 2 | 5.0 | 4 | 4.8 |
| Treated teeth with cellular infiltration | 2 | 6.2 | 0 | 0 | 5 | 12.5 | 7 | 8.4 |
| Treated teeth with pulpal abscess | 3 | 9.4 | 5 | 45.5 | 11 | 27.5 | 19 | 22.9 |
| Treated teeth with delimited necrosis | 13 | 40.6 | 2 | 19.2 | 8 | 20.0 | 23 | 27.7 |
| Treated teeth with total necrosis | 10 | 31.0 | 3 | 27.3 | 9 | 22.5 | 22 | 26.5 |
| Control teeth examined | 16 | .... | 8 | .... | 29 | .... | 53 | .... |
| Control teeth with pulpal pathosis | 0 | 0 | 0 | 0 | 2 | 6.5 | 2 | 3.8 |
| Treated teeth examined periodontally | 35 | .... | 8 | .... | 33 | .... | 76 | .... |
| Treated teeth with normal periodontium | 16 | 45.7 | 1 | 12.5 | 16 | 48.5 | 33 | 43.4 |
| Treated teeth with periodontal infiltration | 7 | 20.0 | 2 | 25.0 | 9 | 27.3 | 18 | 23.7 |
| Treated teeth with periodontal abscess | 7 | 20.0 | 4 | 50.0 | 7 | 21.1 | 18 | 23.7 |
| Treated teeth with periodontal proliferation | 5 | 14.3 | 1 | 12.5 | 1 | 3.0 | 7 | 9.2 |

teeth, the area extended approximately equidistantly into each root (fig. 2 *B*). Cats receiving injections of streptococci showed this reaction more frequently than others.

(*e*) Total necrosis. Total destruction of the pulp occurred in 26.5 per cent of the irritated teeth. The pulp cavity either appeared completely empty, as though it had been filled with a gas or a fluid, or contained a faintly-staining coagulum.

*Control Teeth.*—The tooth opposite the one operated on served as a control in each case. These were subjected to the same preparation as the irritated teeth. Fifty-one of the 53 controls examined showed no pathosis except fibrosis in the teeth of old animals. Two control teeth had a slight generalized infiltration of polymorphonuclear cells.

*Pathologic Changes of the Periodontium.*—In contrast with the 10 per cent of normal pulps found in teeth operated on, 43.4 per cent of

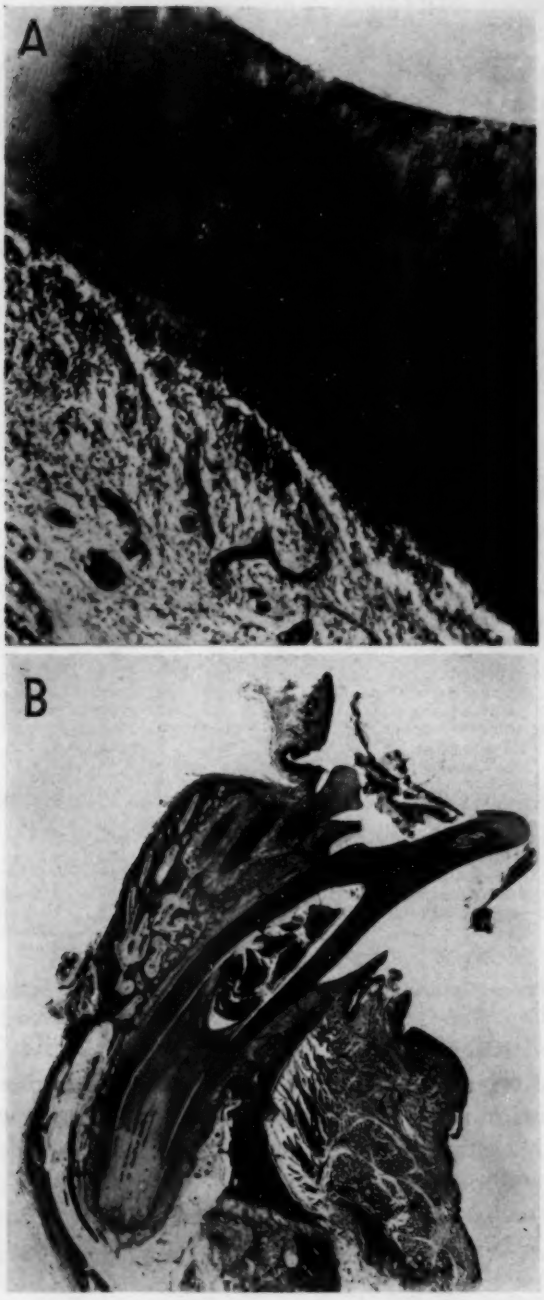


Fig. 1.—*A*, cellular infiltration localized in an area of the pulp near the cavity prepared twenty days before death. Streptococci were injected seven days before death. *B*, delimited necrosis with abscess formation resembling an infected infarct in pulp with a cavity prepared fourteen days before death. Streptococci were injected fifteen days before death.

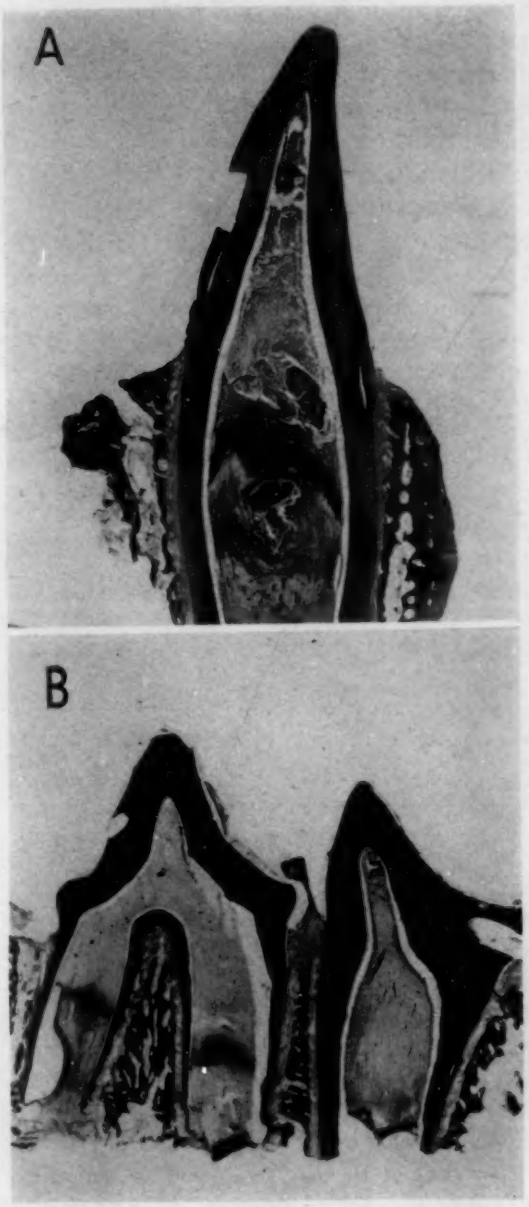


Fig. 2.—*A*, multiple abscesses in pulp with a cavity prepared twenty-seven days before death. Streptococci were injected thirteen days before death. *B*, delimited necrosis resembling an infarct in pulp of a multirooted tooth with a cavity prepared fifteen days before death. The animal was naturally infected through exposed pulp in another tooth. Note also the small abscess at the tip of the tooth on the right, in which a cavity was prepared eight days before death.

76 examined were found to have normal periapical tissue (table 1). The reason for the difference in number of pulps examined (83) and number of teeth examined periapically (76) is that the periapical tissue was lost in some preparations showing the entire pulp.

Eighteen teeth (23.7 per cent) had only leukocytic infiltration of the periodontium, with no other disturbance. The same number presented abscesses in the periapical tissue (fig. 3 *A*).

Proliferation of granulation tissue was found at the apexes of 7 teeth (fig. 3 *B*). In each case this finding was associated with necrosis of the pulp. Bone destruction had occurred to such an extent as to be roentgenographically recorded. Four of the 7 had well formed dental granulomas with marked connective tissue proliferation.

TABLE 2.—*Tissue Reaction in Relation to Period of Infection*

| Tissue Reaction | Teeth | Time from Bacteremia to Death, Days | |
|---|---|---|---|
| | | Average | Mean |
| Pulp normal | 3 | 13 | .. |
| Hyperemia of pulp | 2 | 15 | .. |
| Pulpal infiltration | 2 | 14 | .. |
| Pulpal abscess | 8 | 12 | 10 |
| Delimited pulpal necrosis | 15 | 10.5 | 10 |
| Total pulpal necrosis | 13 | 29 | 30 |
| Normal periodontium | 17 | 15.8 | 13 |
| Periapical infiltration | 9 | 10 | 7 |
| Periapical abscess | 12 | 21.7 | 18 |
| Periapical proliferation | 6 | 32.7 | 30 |

*Relationship of Age and Time Factors to Type of Response.*—The cats were divided into three age groups established by the condition of the root apex observed in sections. Animals whose teeth had open apical foramens were classified as young, those with closed foramens as adult and those with marked hypercementosis of all teeth as old. A chart comparing the reaction to operation shows only two significant differences in type of reaction relative to age of the tooth: a tendency for delimited necrosis to appear more frequently in the teeth with open apexes, and a tendency for abscesses to occur oftener in the older teeth. Although only 27.9 per cent of the teeth examined were from young animals, 52.6 per cent of the teeth showing delimited necrosis were in this group. Twenty per cent of abscessed teeth were in young animals. The large proportion of teeth with delimited necrosis is partially offset by a lower percentage of experiments of long duration on young cats, but since the time relation of abscess formation and the appearance of delimited necrosis is so close (table 2) as compared with the next more destructive reaction, a comparison between these two is still valid. No

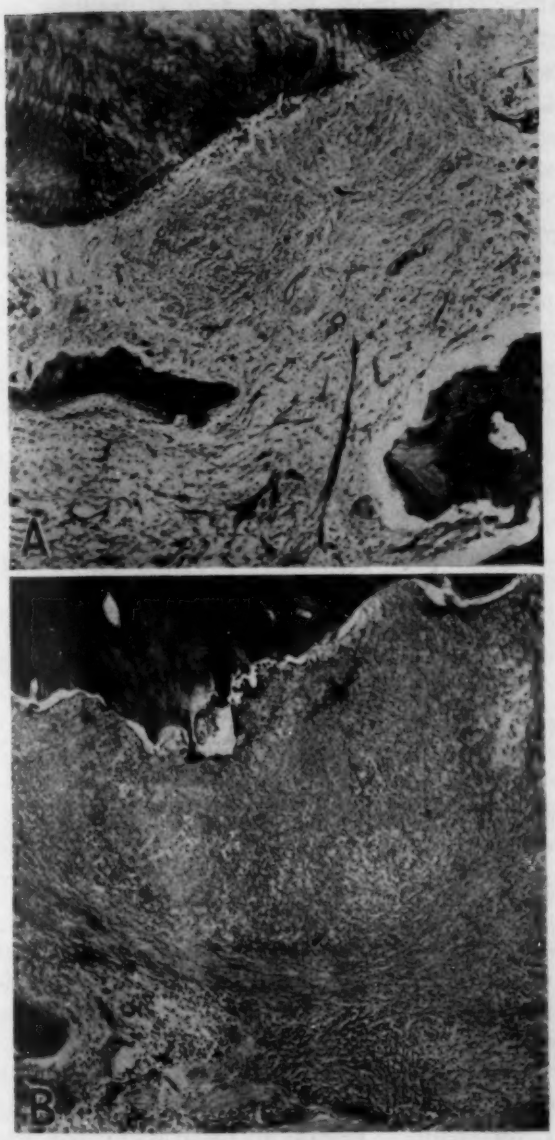


Fig. 3.—*A*, abscess in apical periodontal membrane of a tooth in which a cavity was prepared thirteen days before death. The pulp was completely necrotic. The animal was naturally infected through fracture of another tooth. *B*, connective tissue proliferation forming a dental granuloma in the apical periodontal membrane of a tooth with a cavity prepared twenty-five days before death. The pulp was completely necrotic. The animal was naturally infected through a fracture of another tooth.

relationship could be established between the nature of the reaction and the time elapsed from cavity preparation to death or with the time between cavity preparation and injection of bacteria.

However, when the reactions of the animals given injections of bacteria were separated from those of the animals with evidence of naturally acquired infection, a rather clear relation appeared between type of lesion and time from bacterial injection to death (table 2). Delimited pulpal necrosis and abscess appeared in those animals killed from one to two weeks after injection, each appearing as early as four days and as late as thirty days (1 tooth only). Total necrosis was found in animals killed from two weeks to two months after injection, with 1 necrotic pulp appearing after four days.

Infiltration of the periapical tissue was found, on the average, ten days, periapical abscess twenty-two days (nine to thirty days) and fibroblastic proliferation thirty-three days (four to sixty days) after injection. The tooth with proliferation after four days and that which had complete pulpal necrosis in four days showed distinct evidence of an inflammatory action of a much longer duration, and undoubtedly both should have been eliminated from consideration, with a consequent increase of about five days in the average duration.

It is, of course, impossible to make such a comparison on teeth of animals with natural infections because the time of occurrence of bacteremia cannot be established. However pulpal abscesses were found, on an average, seven days, delimited necrosis fourteen days and total necrosis twenty-seven days after cavity preparation in this group, durations which are comparable to the averages after injection for the same condition in those with bacterial injections. It seems logical to conclude that bacteremia was present at the time of operation or very shortly thereafter.

## COMMENT

The observations on the reaction of dental pulps to irritation followed by or occurring simultaneously with generalized bacteremia indicate a definite sequence. Hyperemia and cellular infiltration are followed within as short a period as one day by abscess formation or the appearance of delimited necrosis or both. If the pulp is unable to overcome these conditions, total necrosis (gangrene) is found, beginning two weeks after bacteremia. Periapical pathosis in our experiment is definitely an extension from the pulp. In all except 1 instance (in a tooth with pulp exposure) the periapical involvement was less marked than the pulp involvement of the same tooth. Abscesses were found about the roots only in teeth with extensive pulpal involvement, and granuloma formation, only in those with complete destruction of the pulp.

There was a noticeable variation in the reaction of individual animals or teeth to the infection. This was undoubtedly due in part to technical variation, as it was impossible to make the cavities of exactly the same depth or distance from the pulp or to control to any extent the length of time the croton oil was in direct contact with the cavity floor.

Slight reactions of hyperemia and simple cellular infiltration were found as late as twenty-five days, and abscess and focal necrosis as late as one month, after the bacteremia was known to have occurred. Since complete necrosis was frequently found at two weeks, it is concluded that the teeth with slighter pathosis after longer intervals were able to overcome or at least resist the destructive processes.

Delimited necrosis was the one reaction observed which might be considered as peculiar to the dental pulp (figs. 1*B* and 2*B*). The resemblance to an anemic infarct was striking. Although engorged venous channels were numerous at the periphery of the necrotic area, a thorough search of a series of sections of each pulp failed to reveal any evidence of thrombosis. This lesion comes nearest to the effect one would expect to find if "self strangulation" were to be the cause of death of the pulp, but in contrast to what would be expected, it occurs most frequently in young teeth with open apical foramens and was found only once in a tooth showing hypercementosis. The possibility remains that in certain cases the hyperemia and subsequent edema of inflammatory response are sufficient to collapse vessels in the crown to such an extent that circulation is inadequate for tissue nourishment as suggested by the authors in 1938.[2] Certainly, such strangulation did not take place first at the apex as earlier writers postulated.[1]

The complete lack of correlation with time of observation after cavity preparation, except in cats with natural infections, compared with the distinct correlation with duration after infection or after cavity preparation in infected animals gives evidence that the irritation in itself is not the cause of pulp destruction. Similarly the high incidence (96.2 per cent) of unaffected control teeth shows that bacteremia alone will not ordinarily cause disease of a healthy tooth. In order to check this premise further, 2 cats were given intravenous injections of 1 cc. doses of a suspension of 130,000,000 staphylococci from a twenty-four hour culture. When examined ten days later the 7 teeth studied histologically and 4 cultured for bacterial growth were entirely without pathologic change. In all cats similarly treated after cavity preparation the teeth operated on showed bacterial growth and disturbance of tissue.

The lapse of time between cavity preparation and induced bacteremia was found to have no effect on the frequency or the severity of the reaction. This lapse varied from immediate injection to twenty days. Resistance is lowered for a considerable period by the treatment given the tooth.

The literature on the subject of attraction of bacteria to inflamed tissue is voluminous and conclusive. A review has been given in the bacteriologic study of this experiment.[4] The source of the bacteremia in the animals given injections and in those with abscesses on the body surface was obvious but the origin of the bacteremia in the animals with simple pulp exposure is more subtle and of greater significance from a dental point of view.

In teeth with pulp exposures complete pulp destruction rapidly developed, with periapical involvement. Bacteremia has been reported in human subjects with oral sepsis by Round, Kirkpatrick and Hails,[5] after mastication of hard candy by Richards,[6] after gingival massage and rocking of teeth by Okell and Elliott,[7] and after extractions by Burket and Burn [8] and Bartels.[9] In the light of these findings it would seem that the effort of chewing hard food (Purina dog checkers) or the manipulation of subsequent operations would furnish sufficient stimulus for the production of transient bacteremia in the cats studied. The bacteremia would lead to infection of pulps which were simultaneously or previously irritated. The mere fact that each cat having a tooth with an exposed pulp reacted in exactly the same way as obviously infected cats is further proof that one infected tooth is a potential source of infection for other teeth if the proper stimulus is provided. In the same way an infection in any part of the body may become the site of origin for destructive infection of a tooth irritated by accident or operative procedure.

## SUMMARY

The teeth of cats irritated by placing croton oil in deep cavities became infected and exhibited varying degrees of pathosis when bacteria were introduced into the blood stream or when infections were present in other parts of the body. No pathosis was observed when bacteremia was present in the absence of irritation.

A definite relation was established between the duration of infection in the presence of irritation and the severity of the resulting lesion. Since no such relation could be established with the duration of irritation, it is concluded that the presence of bacteria is the important factor.

Evidence from the pathologic study and from a previous bacteriologic approach to the same problem indicates that the invading bacteria are blood borne except in cases of pulp exposure or, clinically, when bacteria penetrate dentinal tubules beneath dental caries.

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5. Round, H.; Kirkpatrick, H. J. R., and Hails, C. G.: Proc. Roy. Soc. Med. **29**:1522, 1936.
6. Richards, J. H.: J. A. M. A. **99**:1496, 1932.
7. Okell, C. C., and Elliott, S. D.: Lancet **2**:869, 1935.
8. Burket, L. W., and Burn, C. G.: J. Dent. Research **16**:521, 1937.
9. Bartels, H. A.: Am. J. Orthodontics **26**:366, 1940.

# HEMANGIOMA OF THE SPLEEN

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While sarcoma is recorded as the most common primary malignant tumor of the spleen, hemangioma is the most frequent benign neoplasm. Confusion has been created by attempts to classify the tumors of the spleen. Thus, lymphangioma, cysts, angiosarcoma and malignant endothelioma have all been included under the title of hemangioma. The object of this study is to differentiate true benign hemangioma from the group of seemingly related lesions.

In a search of the literature, only 36 cases of true hemangioma of the spleen could be found. The first case was recorded by Virchow in 1846. Lubarsch, Kaufmann, Trappe, and Krumbhaar and Scott cited instances in which splenic hemangioma was an incidental finding at necropsy. Hodge described a tumor diagnosed as hemangioma which displaced all the splenic tissue. Theile collected 3 cases from Lubarsch's material of 19,000 necropsies. Of 28 splenic neoplasms reported by Krumbhaar in 5,000 autopsies, only 1 was classified as hemangioma. He reported later 40 primary splenic growths, none of which showed angiomatous characteristics.

The occurrence of benign cavernoma in the spleen and in other organs is well known (Homans; Shennan; Dowd; Ugriumow). From the anatomic description of some of the cases it appears that many of the tumors heretofore recorded as benign hemangioma of the spleen were in reality malignant neoplasms. Dowd's summary and classification of 13 cases of hemangioma includes 5 such instances (Langhans; Shennan; Theile; Jores; Ernst). A similar case was later cited by Orlandi.

The present paper deals with 6 new cases of hemangioma of the spleen, which with the cases reported in the literature are reviewed and correlated.

In a series of 3,676 consecutive necropsies at the Jewish Hospital, hemangioma of the spleen was an incidental finding six times. In no case was there clinical evidence that such a lesion was present in the spleen.

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From the Department of Pathology, Jewish Hospital.

REPORT OF CASES

CASE 1.—A woman aged 70 was admitted because of bilateral pneumonia and died several days later after open thoracotomy had been done for empyema on the left side.

The spleen measured 14.5 by 8 by 4.5 cm. and weighed 212 Gm. On its diaphragmatic surface were two depressions, each of which measured 3 mm. in diameter. The spleen revealed in its upper third a circumscribed nonencapsulated honeycombed mass, which measured 4.5 cm. in diameter. The spaces composing the spongelike tissue measured up to 3 mm. in diameter and were filled with dark red blood, occasionally also with a homogeneous gray translucent material. Separating the spaces were bands of fibrous tissue. Section through the depressed areas revealed two circumscribed homogeneous gray nodules, measuring 1 and 1.5 cm., respectively. In the center of one of these nodules were two small cavities filled with brown-red fluid.

Microscopically the tumor in the spleen showed variable pictures. The larger portion consisted of narrow and wide blood spaces, the smallest of which were only a little larger than the normal splenic sinus, while the larger spaces reached a diameter of 3 mm. Other portions of the growth consisted almost entirely of the very smallest sinuses. The spaces were lined in the main by closely placed columnar cells. The fact that the lining cells were closely placed indicated that the wide spaces were not simply dilated splenic sinuses, but neoplastic. Within the cavernous spaces were dense clumps of erythrocytes. In other spaces there was a light staining pink homogeneous material with scattered small aggregations of erythrocytes. This homogeneous substance is often referred to as hematogenous hyalin and is said to arise from red cells and fibrin. There was also found in certain instances a pink fibrinous network in which were scattered polymorphonuclear leukocytes and intracellular and extracellular black-brown pigment. Various combinations of some or all of these elements appeared within the blood spaces.

Separating the cavernous spaces were wide branching bands of fibrous tissue; in areas the latter appeared densely cellular and occasionally also fibrocollagenous. In other areas they were homogeneous and hyalinized.

Portions of the tumor which were composed entirely of the very small sinuses contained little if any blood; these sinuses were lined by thready endothelium. In places the endothelium had been shed into the vascular spaces and assumed the appearance of foam or pseudoxanthomatous cells. Fat stains indicated that the cells contained neutral fat. Round cells, histiocytes and polymorphonuclear leukocytes were found scattered in the tumor tissue. Certain portions of the angioma were replaced by proliferating fibroblasts. It is our opinion that the portions of the tumor composed of the smaller blood spaces probably represent an early stage in the development of angioma, i. e., the stage before hypertrophy and dilatation of the sinuses take place (fig. 1 *A*).

Certain portions of the tumor were infarcted, with partial fibroblastic replacement. There were also isolated areas in the tumor which resembled the normal splenic pulp. They differed, however, from the normal in that the collagenous matrix was more pronounced and the lymphoid elements diminished.

At the periphery the tumor merged with the normal splenic pulp. Occasionally a trabecular artery from the splenic pulp entered the new growth, soon dividing into smaller branches. The trabecular capillaries within the tumor were engorged with blood and stood out in sharp contrast to the surrounding angiomatous spaces, which were relatively empty.

Microscopic examination of the two sclerotic nodules showed loose and dense fibrous tissue. Toward the center the tissue was edematous and embryonal in character. More peripherally the connective tissue merged with what appeared

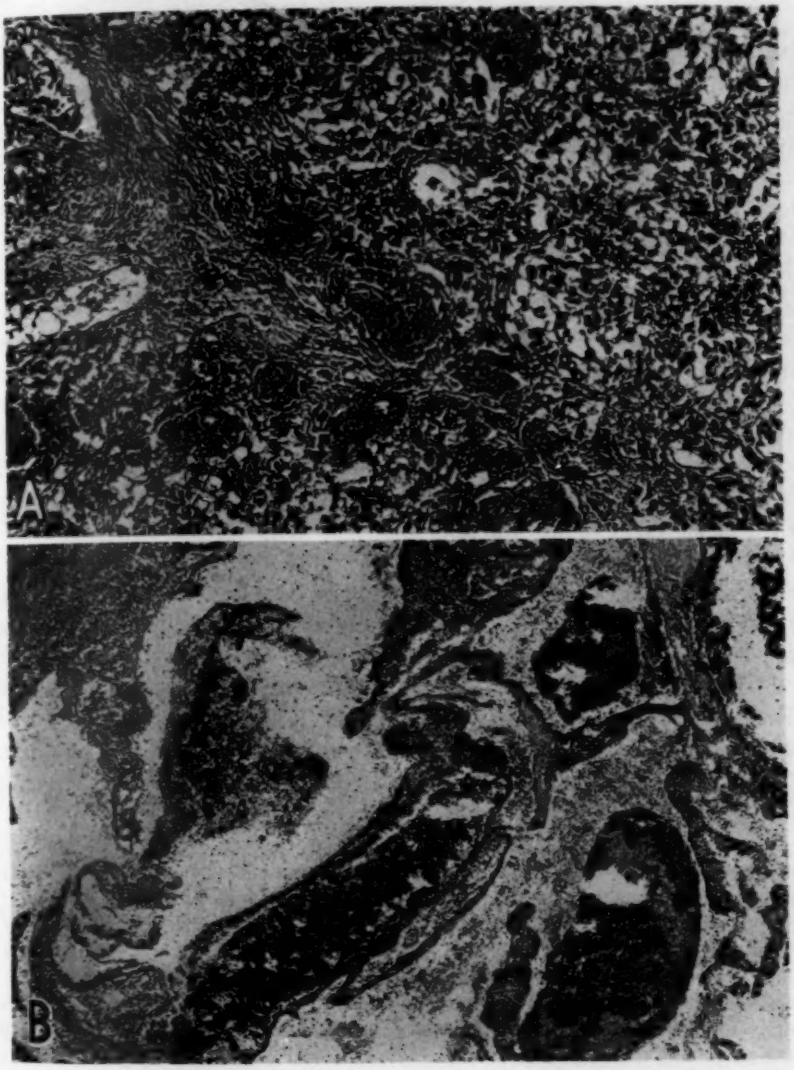


Fig. 1.—*A*, section of the cavernous hemangioma composed of the very smallest sinuses. Note the empty blood spaces lined by thready endothelium and the presence of a splenic trabecula and congested trabecular capillaries (× 270). *B*, hemangioma telangiectoides showing irregular communicating blood spaces containing clumps of disintegrated red blood cells. The tissue between the blood spaces consists of normal splenic pulp (× 40).

as granulation tissue, while the outermost portions were composed of dense collagenous and hyalinized connective tissue. Enclosed within the latter were remnants of angiomatous tissue. The splenic pulp adjacent to the angioma was compressed.

An analysis of this case suggests that early in its development the hemangioma consisted of small endothelial-lined spaces which contained little or no blood. During this phase there appeared to be no continuity between the small and large sinuses and the general splenic circulation. In the subsequent stages, however, circulatory continuity became established, and as a result hypertrophy and dilatation of the blood spaces took place.

While some of the larger arteries adjoining the hemangioma were lined with endothelium of the same type as that lining the hemangiomatous spaces, no direct connection between the two vascular channels could be discerned. However, the finding of direct extension of the splenic arteries into the tumor suggests that such a condition might nevertheless have been present. It is also known that this type of tumor may arise from a capillary, venous or arterial segment of the vascular tree of the spleen. In 1863 Virchow, and since then other investigators, have demonstrated the existence of such vascular connections by injecting a traceable material into the angioma.

Another interesting feature was the infarction of the hemangioma. Evidence of the earlier phases of infarction was seen in the main tumor mass, while the two sclerotic nodules represented the end result of fibrosis and hyalinization.

The apparently normal splenic tissue in the hemangioma indicates that the lesion was not a true neoplasm but rather a malformation of the splenic tissue.

Case 2.—A woman 65 years of age was admitted because of hypertension and splenomegaly and died from cardiovascular insufficiency.

The spleen measured 21 by 14 by 4 cm. and weighed 900 Gm. Toward the upper pole was a small elevated nodule, about 3 cm. in diameter. The cut surface presented three distinct tumor masses, measuring 2.5 cm., 2.4 cm. and 5 mm., respectively; the first of these conformed in outline with the nodule noted on the surface.

With but few exceptions, the three nodules were similar in histologic character to those described in case 1. The capsule surrounding the tumors was composed partly of well formed fibrous tissue and partly of fibroblasts, polymorphonuclear leukocytes and round cells.

There was also found in one area a group of fusiform, round and oval-shaped cells embedded in a hyalinized connective tissue matrix. The stroma contained numerous large histiocytes filled with brown pigment. This lesion is commonly referred to as a Gandy-Gamna nodule. The preponderance of histiocytes and the presence of abundant pigment indicate that this area was the seat of a previous hemorrhage.

CASE 3.—A man 63 years of age had had rheumatic heart disease since the age of 20 and died of congestive heart failure.

The spleen measured 11 by 6 by 3.5 cm. and weighed 70 Gm. The cut surface presented a well circumscribed but nonencapsulated spongy purple nodule. The latter showed features similar to those described in the first 2 cases and requires, therefore, little comment. The sinuses contained in addition to various cellular elements large mononucleated and multinucleated cells with abundant foamy cytoplasm.

CASE 4.—A 4 year old boy died of acute appendicitis and focal pneumonia. At necropsy the spleen weighed 44 Gm. The cut surface showed a circumscribed dark blue-red area, 1.5 cm. in diameter. Microscopically the latter consisted of wide spaces in a matrix of splenic pulp (fig. 1 *B*). The spaces varied in size up to 3 mm. in diameter; they were lined by flat endothelial cells and contained small collections of polymorphonuclear leukocytes, desquamated endothelium and occasional lymphocytes and phagocytes. The latter were laden at times with pigment. Normal splenic pulp was found between the cavernous spaces. The splenic pulp bordering the neoplasm showed degenerative changes and dissolution of the matrix. As a result, the sinusoids were converted into large spaces containing degenerated cells and red and white blood cells. Occasionally these wide spaces appeared to communicate both with the cavernous spaces of the hemangioma and with the normal sinusoids of the spleen.

Morphologically the case differed in certain respects from the 3 cases just described. The lesion was not definitely circumscribed, and the splenic pulp formed the essential supportive tissue for the cavernous channels. Although the general configuration of this lesion appeared similar to that commonly seen in simple cavernoma, nevertheless its basic structure consisted more of an ectasia of the sinusoids, resulting in part at least from an inflammatory process in the pulp.

CASE 5.—A 42 year old woman died of carcinoma of the ovaries with general metastasis. At necropsy the spleen was markedly enlarged and contained a small irregular nonencapsulated blue-black area, which measured 0.5 cm. in diameter and microscopically consisted of wide blood spaces set in a matrix of splenic pulp (fig. 2 *A*). In appearance this area was similar to the angiomatous area described in case 4 except for the absence of an inflammatory reaction. The cavernous spaces were lined with the same type of endothelium as that in the splenic veins, which suggests that this was a capillary or venous ectasia rather than a new growth.

CASE 6.—A 68 year old man died of carcinoma of the adrenal with general metastasis. At necropsy the spleen measured 15 by 10 by 6 cm. and weighed 405 Gm. Near the anterior margin was a conical depression, 1 cm. in diameter. The cut surface of the spleen presented several round gray areas up to 0.2 cm. in diameter. Immediately underlying the conical depression was an irregular blue-red area, which measured 2.2 cm. in diameter. The latter consisted of closely placed intercommunicating blood spaces set in a delicate connective tissue matrix (fig. 2 *B*). The structure and the arrangement of these spaces were strikingly uniform. Each space was slightly larger than the normal splenic sinusoid and was lined by uniformly placed cuboid cells. The spaces contained red blood cells and myelocytes; the latter were conspicuous throughout the entire spleen.

The fibrillar matrix enclosing the sinuses of the angioma was generally thin and indistinct. The collagenous fibers appeared to arise from the splenic trabeculae and from the walls of the trabecular vessels which traversed the angioma. A few splenic corpuscles were noted within the tumor.

The angioma was not encapsulated and merged almost imperceptibly with the splenic tissue. The reticulum of the splenic pulp was continuous with that of

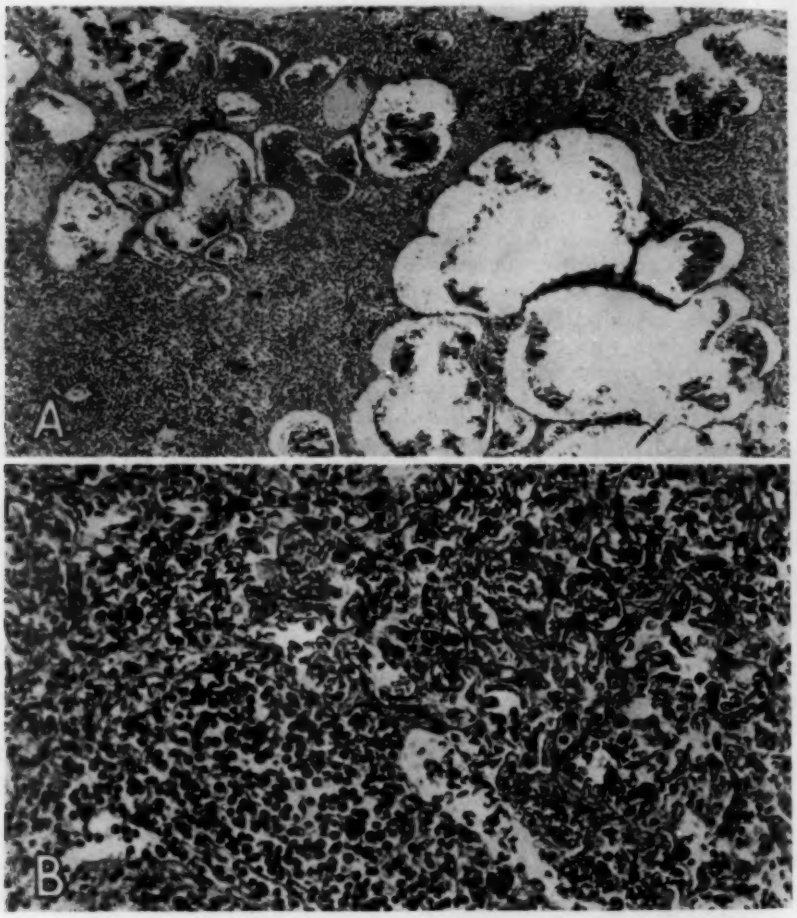


Fig. 2.—*A*, hemangioma telangiectoides; wide blood spaces set in a matrix of splenic pulp (× 80). *B*, hemangioma simplex. Note the uniform intercommunicating spaces lined by cuboid cells. Within the lumens are red blood cells and myelocytes. Note the presence of a splenic corpuscle in the hemangioma.

the tumor matrix. The angioma was distinguished from the normal splenic pulp only because of its uniformly arranged larger sinusoids, the absence of Billroth cords and the paucity of white pulp. The portion of the angioma immediately below the splenic capsule was infarcted.

The structure of the angioma in this case differed in several respects from that in the preceding cases. The blood spaces were small and uniform in size and were lined by an unusually dense layer of cuboid cells. Such a feature is characteristic of capillary angioma. Near the periphery there was a definite transition from the irregular blood spaces of the spleen to the more uniformly arranged spaces of the neoplasm. The transition from the normal splenic parenchyma to hemangioma suggests that we are dealing here with a cellular rearrangement in the anatomic configuration of the splenic parenchyma rather than with the formation of a new growth. This is further supported by the direct vascular continuity between the angiomatous spaces and the sinusoids of the adjoining red pulp. Moreover, the disclosure of normal splenic

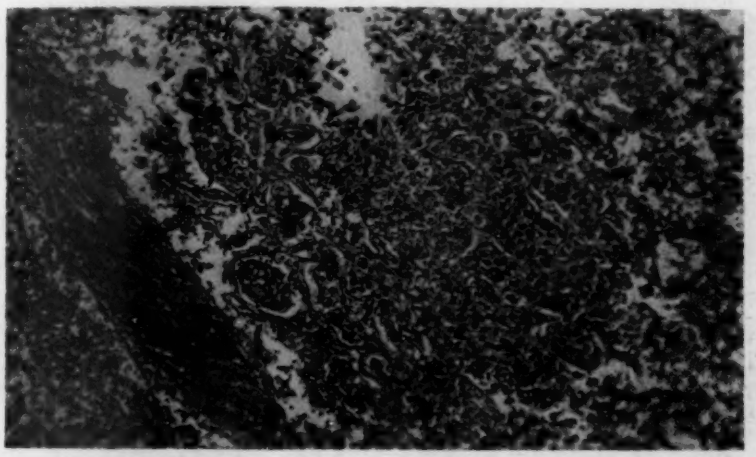

Fig. 3.—Nests of metastatic cells of an adrenal carcinoma filling the sinuses of the hemangioma (× 140).

tissue within the tumor adds further proof that such an anatomic rearrangement occurred.

There was, in this case, metastasis of adrenal carcinoma within some of the angiomatous spaces, red pulp and malpighian corpuscles (fig. 3).

## COMMENT

In order to arrive at a general understanding of hemangioma of the spleen, the 36 cases collected from the literature and our 6 cases were analyzed and tabulated (table). In all there are 42 known cases of hemangioma of the spleen. In 52.4 per cent of the cases the hemangioma was an incidental observation at necropsy; in 47.6 per cent of the cases it was found at operation.

*Analysis of Cases of Hemangioma of the Spleen*

| Author | Sex | Age, Years | Character of Hemangioma of Spleen | Spleen | | Unusual Anatomic Findings in Hemangioma |
|---|---|---|---|---|---|---|
| | | | | Size, Cm. | Weight, Gm. | |
| Virchow | M | 25 | Cavernous, a single nodule | ..... | ..... | ..... |
| Virchow | M | 62 | Cavernous, a single nodule | ..... | ..... | ..... |
| Virchow | F | 72 | Cavernous, a single nodule | ..... | ..... | ..... |
| Hodge | F | 45 | Cavernous, involving whole spleen | 20×12×5.4 | ..... | ..... |
| Homans | F | 22 | Cavernous, involving whole spleen | 15×11×4 | 415 | Areas of thrombosis |
| Lubarsch | M | 53 | Cavernous, single cherry-sized nodule | ..... | ..... | ..... |
| Trappe | .. | .. | Cavernous, a single nodule | ..... | ..... | ..... |
| Albrecht | M | 65 | Cavernous, 6 lentil to pea-sized nodules | ..... | ..... | ..... |
| Theile | M | 22 | Cavernous, 1 cherry-sized nodule | ..... | ..... | Areas of thrombosis and infarction |
| Theile | M | 31 | Cavernous, 2 cherry-sized nodules | 14×7×3 | ..... | Areas of thrombosis and infarction |
| Theile | M | 62 | Cavernous, multiple bean-sized nodules | Normal | ..... | Areas of thrombosis and infarction |
| von Beckendorff | F | 53 | Cavernous, involving whole spleen | 17×8×4.5 | ..... | ..... |
| Martin | F | .. | Cavernous, multiple nodules varying in size from that of a pinhead to that of a 2 franc piece | ..... | ..... | Areas of thrombosis. Histologic picture is that of capillary and sinus ectasia in normal splenic pulp |
| Anzillotti | F | 38 | Cavernous, multiple walnut-sized nodules | 18×11×7 | 1,000 | Areas of thrombosis |
| Dowd | F | 37 | Cavernous, involving whole spleen | 20×12×7 | ..... | Blood cyst 10 cm. in diameter in angioma |
| Müller | M | 30 | Hemangioblastoma, 33 nodules, measuring 0.7-1.2 cm. | 14×13×4 | 260 | Tumors consist of large blood channels, numerous tiny capillaries and uncanalized endothelium |
| Steden | M | 42 | Cavernous, involving whole spleen | 26×21.5×14.5 | 4,125 | Areas of thrombosis |
| Dobrzaniecki | .. | .. | Hemangioma telangiectoides | ..... | ..... | Angioma appears to be the result of dilatation of blood vessels of spleen |
| Naher | F | 42 | Cavernous, involving whole spleen | 24×20×10 | 2,500 | Areas of thrombosis |
| Ugriumow | M | 20 | Cavernous, involving whole spleen | 31×17×9 | 2,300 | Portal cirrhosis found. Histologic picture of angioma is one of capillary ectasia |
| Krumbhaar and Scott | M | 31 | Cavernous, 1 nodule | ..... | ..... | ..... |
| Schwartz | M | 35 | Cavernous, involving whole spleen | 24×18×10.5 | 5,500 | Areas of thrombosis |

| | | | | | | |
|---|---|---|---|---|---|---|
| Wassilijeff and Pratassewitch | M | 32 | Cavernous, involving whole spleen... | 35×16×10 | 2,900 | ..... |
| Kaufmann..... | .. | .. | Cavernous, 1 cherry-sized growth.... | ..... | ..... | ..... |
| Kellert..... | F | 52 | Cavernous, involving whole spleen... | ..... | ..... | Areas of thrombosis and infarction; abscess in an infarct |
| Palavecino and co-workers.... | M | 41 | Cavernous, involving whole spleen... | ..... | ..... | Gandy-Gamna nodule in angioma |
| Haines and McIlroy..... | F | 43 | Cavernous, 1 nodule..... | 10×5×4 | ..... | Rupture of angiomatous space with intra-abdominal bleeding |
| Mikami..... | F | 45 | Cavernous, 12 nodules 0.2-2 cm. in diameter | 12×7×2 | 160 | Gandy-Gamna nodule in angioma |
| Schottenfeld and Wolfson..... | F | 31 | Cavernous, involving whole spleen... | 21×18×10 | ..... | Area of thrombosis and infarction |
| Grove..... | F | 4 mo. | Hemangioblastoma, 1 nodule 5 × 5 cm. in diameter | ..... | 70½ | Angioma consists of proliferating fibroblastic cells and densely placed capillaries |
| Dawson..... | F | 68 | Cavernous, 1 large tumor..... | ..... | ..... | Wandering spleen with torsion of pedicle; areas of thrombosis and infarction in angioma |
| Akcakoyunlu..... | M | 30 | Cavernous, involving whole spleen... | 36×26×12 | 7,240 | Splenic pulp largely replaced by blood-filled capillary and cavernous spaces |
| Nesler and co-workers..... | M | 40 | Cavernous, involving two thirds of spleen | ..... | 125 | Rupture of angiomatous space with intra-abdominal bleeding |
| Goldberg..... | M | 67 | Cavernous, 1 nodule 4 cm. in diameter | 12×10×5 | 265 | ..... |
| Cole and Forsee..... | M | 27 | Cavernous, multiple nodules 0.5-3 cm. in diameter | ..... | ..... | Rupture of angioma of spleen with intra-abdominal bleeding |
| Goldberg..... ..... | F | 45 | Cavernous, involving whole spleen... | 19×14×7 | ..... | ..... |
| Authors' case 1..... | F | 70 | Cavernous, 3 tumors 1-5 cm. in diameter | 14.5×8×4.5 | 212 | Areas of thrombosis and infarction; sclerotic nodules consisting of connective tissue containing remnants of angioma |
| Authors' case 2..... | F | 65 | Cavernous, 3 nodules 2.5 cm. in diameter | ..... | 900 | Areas of thrombosis and infarction and Gandy-Gamna nodules |
| Authors' case 3..... | M | 63 | Cavernous, 1 nodule 1.5 cm. in diameter | 11×6×3.5 | 76 | Foam cell collections in angiomatous spaces giving pseudoxanthomatous appearance |
| Authors' case 4..... | M | 4 | Hemangioma telangiectoides, 1 nodule 1.5 cm. in diameter | ..... | 44 | Histologic picture is that of sinus dilatation secondary to a degenerative process |
| Authors' case 5..... | F | 42 | Hemangioma telangiectoides, 1 nodule 4 mm. in diameter | ..... | 420 | The picture is one of ectasia of the splenic veins |
| Authors' case 6..... | M | 68 | Angioma simplex, 1 nodule 1 cm. in diameter | 15×10×7 | 405 | Capillary angioma with areas of infarction and sclerosis; carcinomatous metastasis into the splenic pulp and into the angioma |

*Sex and Age.*—Of the 42 patients, 19 were females and 20 males; in 3 instances the sex was not recorded. The average age of the patients was 42.2 years; the youngest was a 4 month old infant and the oldest a 72 year old man.

*Size, Weight and Rate of Growth.*—In the necropsy group of cases, the spleen was of relatively normal size and the hemangioma varied in size from 1 mm. to 4.5 cm. in diameter. In 7 of the 22 cases there were multiple tumor nodules in the spleen. In the operative group, the spleen was decidedly large; the recorded weight varied from 415 to 7,240 Gm. The largest specimen (Akcakoyunlu) measured 36 by 26 by 12 cm. In only 3 instances was the spleen reported as small (Haines and McIlroy; Nesler, Faber and Leik; Cole and Forsee). Surgical intervention was undertaken in the last 3 cases because of hemorrhage following rupture of the hemangioma.

Hemangioma in the removed spleens was mostly a single large growth which involved the entire or the major portion of the organ. Clinical examination disclosed a smooth mass in the left upper quadrant of the abdomen. In Dawson's case the tumor was palpated in the left lower quadrant and was taken for an ovarian cyst.

Thirteen of the surgically removed specimens came from females. The preponderance of the larger forms of hemangioma in females may be attributed to anatomic and physiologic causes. Possibly hormonal stimulation of the vascular system may occur during menstruation or gestation; or compression of the splenic vessels by an enlarged uterus may cause distention of the blood spaces in already existing hemangioma of the spleen. The increase in blood pressure will favor the formation of new channels in the neoplasm. Follin described a tumor in the lip, diagnosed as cavernous angioma, which was influenced by menstruation and grew with pregnancy. We observed a flat hemangioma of the neck in a woman for many years. It began to grow with the onset of pregnancy and at parturition had become a large pedunculated mass. Similarly, Owen described angioma of the face in a woman, which had been present since the age of 7; the tumor showed more rapid growth after the onset of menstruation.

A vascular growth of the spleen may remain quiescent for many years. It may enlarge slowly and cause gradual compression of the adjacent organs, or it may grow rapidly. The duration of symptoms in 10 of the operative cases gives a clue to the rate of growth. The average duration of symptoms was four and three-tenths years. In 1 case the lesion was present for twenty years before splenectomy became necessary (Palavecino and co-workers).

Obviously angioma of the spleen may be present for a considerable length of time before symptoms are produced. The topographic anatomy

and physiology of the spleen is such that symptoms do not occur unless the organ is of sufficient size to impinge on neighboring organs.

*Alterations of Hemangioma.*—Hemangioma of the spleen may show regressive changes. Thrombosis and infarction occur in many of the cases because of the limited vascularity of the tumor. These alterations were found in 38 per cent of the cases. When bacteria gain entry into an infarct, abscess may develop. Kellert recovered streptococci from an abscess within an infarcted tumor diagnosed as hemangioma. Since the central portion of hemangioma is the first to become infarcted, it follows that fibrosis often proceeds from the center toward the periphery of the tumor. Theile, Albrecht and others have described broad central fibrous bands within the tumor. This was also found in our first case. When completely organized, infarcted hemangioma may resemble fibroma or a healed simple infarct of the spleen. Two sclerotic nodules of healed hemangiomatous infarcts were described in case 1.

Occasional calcification of an organized infarct may be seen. Lubarsch cites 2 cases in which this occurred and von Benckendorff another. A small calcific plaque in an area of scar tissue was also noted in case 1. Hemorrhage within hemangioma is uncommon; it occurred in 5 of the cases recorded in the literature.

Cystic changes may occur in hemangioma of the spleen in a number of ways: (1) from liquefaction of a hemorrhagic or necrotic area; (2) from shutting off of a dilated sinus from its efferent vessel, the vis a tergo of the afferent vessel producing dilatation of the sinus; (3) from formation of a cyst in the splenic parenchyma adjoining the tumor, the cyst later breaking into the tumor. Billroth described the first cystic hemangioma. Fowler collected 43 cases of hemorrhagic cysts of the spleen, in only 2 of which the condition was of angiomatous origin. Dowd recorded an angioma containing a blood cyst that measured 10 cm. in diameter, and Dawson reported a large cystic cavernoma.

Benign hemangioma may become malignant, invade the adjacent parenchyma, break into the blood vessels and metastasize to other organs. Theile was able to demonstrate in the same specimen all morphologic gradation from simple benign cavernoma, through early proliferative changes, up to frank and unquestioned spindle cell sarcoma. Langhans, Orlandi and Wright reported cases of angioma showing malignant endothelial proliferation. These cases also showed various morphologic gradations from differentiated benign endothelium to undifferentiated malignant proliferating endothelium. Smith and Rusk collected 7 cases of angioma of the spleen which showed sarcomatous changes. An extravascular form of malignant endothelial tumor was cited by Jopson. Schlopsnies reported a neoplasm diagnosed as malignant hemangioma involving the spleen, liver and bone marrow. He considered this to be a

diffuse systemic malignant growth which involved the entire endothelial system and which he termed "angioma sarcomatoides systematicum."

Some writers make no distinction between malignant angiosarcoma, endothelial sarcoma and angioma of the spleen. Although these various growths all take their origin from vascular or sinus endothelium, they show no similarity in their clinical or morphologic manifestations. According to Smith and Rusk, endothelioma, endothelial sarcoma and angiosarcoma are malignant counterparts of angioma. In endothelioma the endothelium merely proliferates in a connective tissue matrix without undergoing angiomatous changes.

Schridde stated that despite its benign structure, hemangioma may metastasize to other organs, especially to the liver. The growth is carried to other organs as a "benign structure" via the blood stream and not through the lymphatics. Borrman, Shennan, Ewing and others have subscribed to this idea. Ewing observed, however, that some of these growths exhibited peculiar features of malignant growth and terminated fatally. It has also been noted that while the primary growth remained benign, the metastasis showed malignant changes. Ward and Jones described a patient who had cavernous hemangioma of the palm for many years. Following direct trauma the tumor began to grow rapidly. The growth was excised but recurred several weeks later, and the epitrochlear and axillary glands became enlarged. The recurrent tumor of the palm was histologically benign, while the growth in the excised nodes was unquestionably malignant. In none of the reported cases of "benign metastasizing hemangioma" of the spleen was there any study of serial sections to determine whether the entire angioma or its metastatic deposits did not show malignant changes. There is no evidence in the literature to establish that metastasis of benign angioma to other organs occurs. The finding of multiple lesions of the same nature in various parts of the body may be explained on the basis of a systemic vascular condition, rather than on that of metastasis.

Metastasis of a malignant growth into a hemangioma of the spleen has never been reported in the literature. Our case 5 showed such a metastasis from an adrenal carcinoma.

Some investigators (Matas; Grove) have expressed the belief that hemangioma increases the functions of the spleen. It is true that a large vascular growth increases the blood capacity of the tissues involved, but there usually would be retardation of the flow of blood through the hemangioma, which would permit stasis, thrombosis and infarction to develop. The splenic parenchyma adjoining the hemangioma is usually compressed and its circulation thereby diminished; this in turn leads to diminution in the activity of the organ. Metabolism and storage of iron within the spleen are disrupted, since the reticular elements are either

diminished or completely absent in hemangioma. Furthermore, the hemopoietic function and the antibody production would be adversely affected in the presence of a tumor which both compresses and replaces the normal splenic pulp.

*Pathogenesis.*—A review of the literature reveals a wide discussion of the genesis of benign hemangioma. Ribbert was of the opinion that hemangioma was an independent proliferation of embryonal angioblastic buds originating in a misplaced germinal area, which subsequently differentiate into neovascular growths. Schmieden suggested that hemangioma forms as a result of an imperfect vascular anlage. If the caliber of the capsular veins is reduced so as to hinder the outflow of blood, there will result stasis and dilatation of the vessels with angiomatous formation. Albrecht arrived at the same conclusion.

The prenatal factors which are held responsible for hemangioma are numerous. The neural theory suggests some unexplained injury to certain segments of the spinal cord or spinal ganglions which may produce changes in the vascular anlage of the corresponding segments. The physical or mechanical theory maintains that pressure on the fetus causes primary local anemia, followed by paralytic hyperemia and ultimately by nevus formation. These impressions, however, are too speculative to warrant serious consideration.

Although some congenital abnormality may be responsible for a predisposition to angioma, the actual development is the result of changes in the vascular system or in its immediate environment. Virchow believed that progressive hyperplasia of an embryonal character was initiated by a local disorder of nutrition of preexisting vessels. Thomas suggested that angioma is due largely to mechanical factors, such as increasing intravascular tension. Such mechanical agents tend at first to excite new growths of vessels, followed later by dilation of these vessels. It is generally recognized, however, that stasis and dilation of vessels, be it local or general, does not necessarily have a congenital basis. Ribbert believed that telangiectasia may assume the form of local growths and show no continuity with surrounding capillaries. Kaposi showed that in the skin of aged persons trauma, inflammation and alcoholism may be followed by vascular proliferation. Langhans believed that in his case of angiosarcoma of the spleen the neoplasm began as simple hemangioma, the result of venous dilatation secondary to hypertension. Ugriumow reported a case of cavernous angioma of the spleen which followed portal cirrhosis.

A satisfactory all-embracing explanation of the development of angioma of the spleen is yet to be found. Matas, Borst, Ziegler, and Delafield and Prudden maintain that hemangioma is composed of the same cellular elements which give rise to primordial blood vessels. Such

a concept presupposes that true angioma must show evidence of angioblastic proliferation, which is characterized by the formation of syncytial masses of cells, cords of cells or capillary buds; the latter may or may not show central canalization, depending on its phase of development. A general survey of the literature reveals only 2 authentic cases of hemangioma of the spleen which present such a picture (Müller; Grove). Similar forms of hemangioma have also been described by Cushing and Bailey in tumors arising from the cerebral blood vessels. They also distinguished true cerebellar hemangioma, which they termed hemangioblastoma, from an allied group which develops as a result of vascular malformation and which is found elsewhere in the brain. Orsos and Schmidt have described similar benign angioma in the nasal mucous membrane, in the jaws and in the legs. They called this lesion "hemogemmangioma"—gemma indicating the predominance of buds. One type of "gemmangioma" which Orsos described consisted of closely placed tiny empty capillaries set in a matrix rich in fibrillar connective tissue. In histologic appearance this tumor was similar to the hemangioma of the spleen described by Grove.

In some cases hemangioblastoma and "hemogemmangioma" at certain stages of their development cannot be differentiated microscopically from their malignant counterpart, angiosarcoma. No absolute morphologic criteria have as yet been established by which it is possible to determine when these growths cease to be benign.

After studying the cases recorded in the literature and those now reported, it seems fair to assume as a working hypothesis that (1) only 2 of the vascular tumors cited in the literature are true growths, (2) that the remaining 40 are in all probability due to faulty development and that (3) the variations encountered in the latter group depend on the stage of vascular development in which the aberration from the normal occurred. Ewing and others agree that in many of these tumors it is difficult to recognize any of the features characteristic of new growth. The presence of small remnants of unaltered splenic tissue within the tumor and the finding of normal splenic pulp as the sole supporting tissue of the tumor add further support to the contention that the great majority of these lesions are not true neoplasms but represent some rearrangement in the normal cytologic pattern of the spleen. It is of interest to note that such were the findings in all of our cases and also in 21 of the 36 cases reported in the literature. In 3 of our cases one could definitely trace a transition from the normal splenic sinuses to those of the hemangioma. Such observations suggest that angioma is formed as a result of alteration in the normal configuration of the preexisting vascular channels of the spleen. This idea of its formation is also upheld by Martin, Ugriumow and Dobrzaniecki. In our case 3 an inflammatory

and degenerative process appeared to produce widening of the preexisting splenic sinuses; in our case 4, phlebectasia or dilatation of the capillaries and sinuses of the splenic pulp was the apparent etiologic factor. Similar changes were also observed by von Benckendorff and Martin. The capillary spaces of the angioma in case 5 appeared to have formed as a result of rearrangement of the endothelial cells of the normal splenic pulp.

*Classification.*—In 1863 Virchow divided cases of hemangioma into three distinct groups, namely, (1) hemangioma simplex, (2) cavernous hemangioma and (3) hemangioma telangiectoides. To these three must be added a fourth group, the cases of so-called angioblastoma or "hemogemmangioma."

Hemangioma simplex includes growths composed of relatively closely packed, uniformly distributed small blood spaces. They show little or no connective tissue stroma. The cells lining the spaces form a uniform continuous layer of cuboidal and cylindric endothelium. The site of predilection is in superficial structures, viz., the skin and the mucous membranes. They are rarely found in the internal organs. Of all the known cases of hemangioma of the spleen, only 1 belonged in this group ~~(our case 6).~~

Cavernous hemangioma consists of freely intercommunicating dilated spaces supported by a variable amount of connective tissue; the latter may also contain some elastic and smooth muscle fibers. Tumors of this type are mostly found in the skin, the orbit, the nose and the pharynx; they also occur occasionally in internal organs. ~~Thirty-three~~ of the collected cases in the literature ~~and 3 of our cases~~ of splenic hemangioma belonged in this group.

Hemangioma telangiectoides or angioma hypertrophicum simplex includes a large group of cases in which the essential lesion is characterized by dilatation of preexisting vessels. The dilated spaces may consist of dilated capillaries or of large aggregates of intercommunicating veins and arteries, or of combinations of all these structures. In only 1 reported case belonging to this group was the tumor in the spleen; such a location was seen in 2 of our cases.

Angioblastoma or "hemogemmangioma" includes the true forms of angioma. The characteristic feature of this type is the presence of angioblastic proliferation, denoting a true neovascular formation. Two such cases have been recorded in the literature in which a tumor of this type occurred in the spleen.

## SUMMARY

After a survey of the literature we were led to believe that only 36 authentic cases of hemangioma of the spleen had been reported. These with the 6 cases which form the basis of this report have been analyzed and divided into four distinct groups.

Group 1 includes 36 cases in which the tumor was classified as cavernous hemangioma. Group 2 includes 1 case in which it was classified as angioma simplex. Group 3 includes 3 cases in which it was classified as hemangioma telangiectoides, and group 4 includes 2 cases in which it was classified as hemangioblastoma or "hemogemmangioma."

Neovascular lesions of the spleen are slow growing and in many instances remain small and undiagnosed throughout life. In 40 per cent of the collected cases the tumor grew to large proportions. Clinical evidence of this lesion was apparent only when it was large enough to produce mechanical compression of the adjacent organs.

Hemangioma of the spleen is a benign lesion and therefore does not metastasize. The presence of the growth in other organs besides the spleen is explained on the basis of a constitutional neovascular disturbance.

It is believed that some congenital anomaly is primarily responsible for the development of this tumor. The actual formation of the growth, however, is due to factors inherent in the vascular system of the spleen itself or of its immediate environment.

Tumor metastasis from another organ to a tumor of the spleen classified as hemangioma is a rare occurrence. In 1 case in our series a tumor of the spleen diagnosed as hemangioma showed metastatic nests from a tumor of the adrenal diagnosed as carcinoma.

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## BIBLIOGRAPHY

Akcakoyunlu, I.: Am. J. Surg. **41**:519, 1938.

Albrecht, H.: Ztschr. f. Heilk. **23**:97, 1902.

Anzillotti, D.: Sperimentale, Arch. di biol. (supp.) **67**:161, 1913.

von Benckendorff, E.: Virchows Arch. f. path. Anat. **194**:500, 1908.

Billroth, T.: Die allgemeine chirurgische Pathologie und Therapie, Berlin, G. Reimer, 1869.

Borrman, R.: Beitr. z. path. Anat. u. z. allg. Path. **40**:372, 1906.

Borst, M.: Histologische Pathologie, ed. 2, Leipzig, F. C. W. Vogel, 1926.

Cole, F. L., and Forsee, J. H.: Surgery **8**:639, 1940.

Cushing, H., and Bailey, P.: Tumors Arising from Blood Vessels of the Brain, Springfield, Ill., Charles C. Thomas, Publisher, 1928.

Dawson, J. B.: New Zealand M. J. **36**:393, 1937.

Deelman, H. T.: Het metastatisch carcinom van longen en milt, Amsterdam, Antoni van Leeuwenhoek-Huis, 1918; cited by Yokohata, T.: Ztschr. f. Krebsforsch. **25**:32, 1927.

Delafield, F., and Prudden, A.: A Textbook of Pathology, ed. 16, Baltimore, William Wood & Company, 1936.

Dobrzaniecki, W.: Zentralbl. f. Chir. **53**:1940, 1926.

Dowd, C. N.: Ann. Surg. **62**:177, 1915.

Ernst, P.: Verhandl. d. deutsch. path. Gesellsch. **15**:232, 1912.

Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1928.

Follin, F. A.: Traité élémentaire de pathologie externe, Paris, G. Masson, 1861.
Fowler, R. H.: Ann. Surg. **57**:658, 1913.
Goldberg, S. A.: Am. J. Clin. Path. **10**:700, 1940.
Grove, L. W.: Ann. Surg. **105**:969, 1937.
Haines, C. E., and McIlroy, P. T.: J. A. M. A. **100**:1862, 1933.
Henke, F., and Lubarsch, O.: Handbuch der speziellen Pathologie und Histologie, Berlin, Julius Springer, 1927, vol. 2, p. 699.
Hodge, M. D., Jr.: M. Rec. **48**:418, 1895.
Homans, J. H.: Ann. Surg. **25**:732, 1897.
Jopson, J. H.: S. Clin. North America **1**:235, 1921.
Jores, L.: Zentralbl. f. allg. Path. u. path. Anat. **19**:662, 1908.
Kaposi, M.: Arch. f. Dermat. u. Syph. **4**:265, 1872.
Kaufmann, E.: Lehrbuch der speziellen pathologischen Anatomie, Berlin, Walter de Gruyter, 1931, vol. 2, p. 228.
Kellert, E.: Am. J. Cancer **16**:412, 1932.
Krumbhaar, E. B.: Pathology, New York, Paul B. Hoeber, Inc., 1937.
Krumbhaar, W. B., and Scott, J. P.: S. Clin. North America **7**:61, 1927.
Langhans, T.: Virchows Arch. f. path. Anat. **75**:273, 1879.
Lubarsch, O.: Ergebn. d. allg. Path. u. path. Anat. **10**:845, 1905.
Martin, A.: Rev. de gynec. et de chir. abd. **13**:67, 1902.
Matas, R.: Vascular Tumors, in Piersol, G. M., and Bortz, E. L.: The Cyclopedia of Medicine, Philadelphia, F. A. Davis Company, 1934, vol. 12, p. 843.
Mikami, M.: Keijo J. Med. **4**:53, 1933.
Müller, H.: Virchows Arch. f. path. Anat. **238**:76, 1922.
Naher, H.: Deutsche Ztschr. f. Chir. **191**:87, 1925.
Nesler, A. B.; Faber, L., and Leik, D. K.: J. Iowa M. Soc. **29**:566, 1939.
Orlandi, N.: Virchows Arch. f. path. Anat. **269**:153, 1928.
Orsos, F.: Beitr. z. path. Anat. u. z. allg. Path. **93**:121, 1932.
Owen, R. H.: South. Med. & Surg. **101**:364, 1939.
Palavecino, I.; Rodríguez Villegas, R., and Palazon, J. M.: Semana méd. **2**:82, 1932.
Ribbert, H.: Virchows Arch. f. path. Anat. **151**:381, 1898.
Schmidt, H.: Frankfurt. Ztschr. f. Path. **51**:43, 1938.
Schmieden, V.: Virchows Arch. f. path. Anat. **161**:373, 1900.
Schlopsnies, W.: Virchows Arch. f. path. Anat. **274**:85, 1930.
Schottenfeld, L. E., and Wolfson, W. E.: Arch. Surg. **35**:867, 1937.
Schridde, in Aschoff, L.: Lehrbuch der pathologischen Anatomie, Jena, Gustav Fischer, 1913.
Schwartz, F.: Beitr. z. klin. Chir. **150**:130, 1930.
Shennan, T.: J. Path. & Bact. **19**:139, 1914.
Smith, S. C., and Rusk, S. J.: Arch. Surg. **7**:371, 1923.
Steden, E.: Arch. f. klin. Chir. **130**:617, 1924.
Theile, F. W.: Virchows Arch. f. path. Anat. **178**:296, 1904.
Trappe, M.: Frankfurt. Ztschr. f. Path. **1**:109, 1907.
Ugriumow, B.: Virchows Arch. f. path. Anat. **259**:366, 1926.
Virchow, R.: Die krankhaften Geschwülste, Berlin, A. Hirschwald, 1863, vol. 3.
Ward, G. E., and Jones, A. G., Jr.: Arch. Surg. **36**:330, 1938.
Wassilijeff, A. A., and Pratassewitch, A. W.: Virchows Arch. f. path. Anat. **279**:79, 1930.
Wright, A. W.: Am. J. Path. **4**:407, 1928.
Ziegler, E.: Lehrbuch der allgemeinen und speziellen pathologischen Anatomie, ed. 11, Jena, Gustav Fischer, 1905, vol. 1, p. 423.

# HISTOLOGIC STUDIES OF THE TISSUES OF RATS FED A DIET EXTREMELY LOW IN SODIUM

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Several years ago the effects of sodium deprivation on rats were reported from this laboratory by Orent-Keiles, Robinson and McCollum.[1] The changes noted were retardation of growth, lesions in the eyes and disturbances in reproductive function. Only histologic examination of the eyes was carried out. The present report deals with a detailed microscopic study of all the tissues of rats placed on the same diet, which contains only 0.002 per cent sodium. The ocular apparatus has also been restudied in an attempt to gain some idea of the genesis of the changes observed.

## MATERIALS AND METHODS

The basal experimental diet used in these studies has been reported by Orent-Keiles, Robinson and McCollum.[1] Its percentage composition is as follows: acid-washed, alcohol-extracted casein, 18.0; methyl alcohol–gaseous hydrochloric acid extract of yeast equivalent to dry yeast, 5.0; sucrose, 67.7; sweet butter fat, 5.0; salts (sodium free), 5.3, and viosterol 15 drops per kilogram. This diet contains 0.002 per cent sodium. The control diet was the same except that it was supplemented with sodium so as to contain 0.66 per cent of this element.

Twenty-nine rats about equally divided as to sex were used. They were placed on the diets when 28 days of age. The deficient and control diets were given some of the animals ad libitum; as to the others, the food intake of the control animals was restricted so as to be equal to that of the sodium-deficient rats. After being on the deficient diet for fifty-nine and eighty days, some of the animals of the ad libitum fed group were given the control diet containing 0.66 per cent sodium for twenty-one days in order to observe their growth response and any changes in the tissues. Rats were examined after being on the diets for periods of from fifty-nine to one hundred and forty-two days.

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1. Orent-Keiles, E.; Robinson, A., and McCollum, E. V.: Am. J. Physiol. **119**:651, 1937.

The animals were killed with ether and autopsies made immediately. Tissues were placed in Zenker-formaldehyde solution.[2] After dehydration, blocks were embedded in paraffin, cut and stained with hematoxylin and eosin.

## GROSS APPEARANCE OF THE ANIMALS

As was expected from the previous study of Orent-Keiles, Robinson and McCollum,[1] after the first few weeks the deficient animals grew much more slowly than their ad libitum fed controls (fig. 1). In general, after eight to ten weeks all animals on a diet low in sodium began to lose weight or failed to gain. However, when after eighty days on the

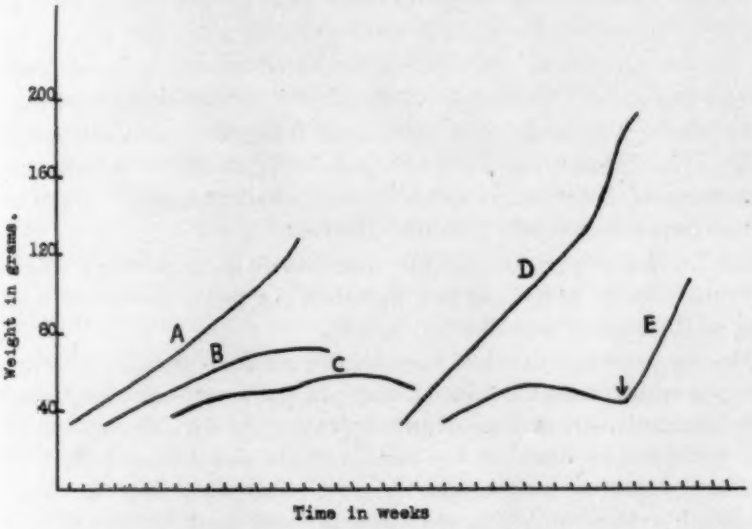


Fig. 1.—Growth curves of groups of animals on deficient and control diets: *A*, paired feeding group on control diet for ninety-nine days; *B*, paired feeding group on deficient diet for ninety-nine days; *C*, ad libitum fed group on deficient diet for one hundred and one days; *D*, ad libitum fed group on control diet for one hundred and one days; *E*, ad libitum fed group on deficient diet for eighty days and then on control diet (at arrow) for twenty-one days.

deficient diet, as shown in figure 1 *E*, rats were given the control diet containing 0.66 per cent sodium, the growth response was rapid, and in three weeks they had gained 59 Gm. In the paired feeding group, despite the fact that the controls received the same amount of food which the deficient rats ate, the former group showed the better growth curve.

Characteristic changes in the eyes also made their appearance between the eighth and tenth weeks. The corneas became cloudy and opaque.

2. This is Zenker's solution prepared with solution of formaldehyde U. S. P. instead of acetic acid.

The lids became swollen and lost their hair. There was a reddish crust about the margins.

At autopsy there was a loss of subcutaneous fat in the sodium-deficient animals. All the organs were naturally smaller; this was especially prominent as regards the thymus and reproductive organs. At one hundred and forty-two days there were small abscesses in the lungs of 2 of the deficient animals.

### MICROSCOPIC OBSERVATIONS

*Alimentary System.*—The lining of the esophagus, the stomach and the intestine was entirely normal. Sections of the salivary glands, the liver and the pancreas likewise showed no change.

*Circulatory System.*—Sections of the heart and of the blood vessels throughout the body showed no change in the sodium-deficient animals.

*Respiratory System.*—The epithelium lining the nares showed no change. The trachea was lined by ciliated epithelium. The pulmonary parenchyma of 2 deficient animals showed small abscesses. These are not uncommon in rats of this colony, however.

*Genitourinary System.*—In the sodium-deficient rats there was no observable change in the kidneys, including the pelvic epithelium. The lining of the bladder was likewise normal.

The testes of rats that had been on the deficient diet fifty-nine days contained smaller tubules than did those of the control animals, though spermatogenesis was active. However, by eighty days formed spermatozoa could not be found in the tubules of the deficient animals, while they were numerous in the controls. At ninety-nine days the tubules were lined by spermatogonia, and a few primary spermatocytes could be seen. In contrast, in the control rats on the paired feeding regimen the tubules were larger and spermatogenesis was taking place. At one hundred and forty-two days the testes of the deficient animals were completely inactive save for occasional mitotic figures. On the other hand, active spermatogenesis was taking place in their paired feeding controls. Differences were also noted in the accessory sex glands of the deficient and control animals. In the former group, the organs were smaller, their epithelial cells were less columnar and secretory activity was much less marked than in the latter animals.

In the ovaries of all the deficient animals there were fresh corpora lutea as well as developing ova. However, they were fewer and smaller in the deficient rats than in their controls. In the uteri of the deficient animals, the epithelial cells were columnar, and some showed evidence of secretory activity. The vaginal epithelium was multilayered in the deficient rats. The lumen was filled with keratinized cells and leukocytes. The latter were found infiltrating the epithelium as well.

*Nervous System.*—Routine sections of the brains from the sodium-deficient groups when stained with hematoxylin and eosin showed no lesions. The peripheral nerves too showed no change.

*Myeloid and Lymphoid Tissues.*—In the medullary cavities of the tibias of the deficient animals there was definitely more fat and less myeloid tissue than in the tibias of their paired feeding controls. The lymphoid tissue throughout the body too was more atrophic. The thymus glands of the deficient group showed the usual atrophic changes seen in inanition.

*Muscle Tissue.*—No changes were noted in the striated and involuntary muscle fibers of the deficient rats.

*Skeletal System.*—Sections were made through the cartilage-shaft junctions of the tibias. There was virtually complete cessation of growth in the deficient animals. The zone of hypertrophic cartilage cells was narrow, and there was practically no bone being laid down at the cartilage-shaft junction. Bone formation was taking place in the paired feeding control group, but it too was greatly retarded. In comparison those animals which had been on the deficient diet and had then received the control diet for three weeks showed greatly accelerated growth activity, which was even more marked than that of the animals which had been on the control diet during the entire experiment.

*Ductless Glands.*—The thyroid glands of the deficient group had smaller follicles than those of the controls. The epithelium, however, showed no change. The parathyroid glands in the two groups of rats showed no differences.

The adrenals of the animals on sodium-low diet were not significantly different from those of the paired feeding controls. The cortices had the same thickness, and the cells in the deficient group appeared entirely normal.

*Skin.*—No change except a decrease in subcutaneous fat was noted in the skin of the deficient animals as compared with the controls.

*Ocular Apparatus.*—The appearance of the lesions in the eyes occurred somewhat irregularly; so the following description is an average of the changes seen from the beginning to the end of the observation period.

In the lids the initial change was a dilatation of the ducts of the tarsal or meibomian glands (fig. 2). This appeared about the ninth to the twelfth week, and as time went on, the ducts became wider and wider and their epithelial lining became flatter. No changes in the acinar cells were noted in the early stages, but at the end of the experiment, after the animals had been on the deficient diet for twenty weeks, the glandular elements showed atrophy of individual cells and pyknosis of some of the cell nuclei. Attached to the lid margins there was a pink-staining

granular material, and it is probable that this blocked the ducts and so caused their dilatation. There was no squamous metaplasia of the duct epithelium. The enlargement of the ducts accounts for the swelling of the eyelids noted during life. From the tenth to the twentieth week the epithelium lining the inner margin of the lid changed its character too. Normally it shows a basal layer of columnar cells, on which are cuboid cells. Lying between are large vacuolated goblet cells. This lining was

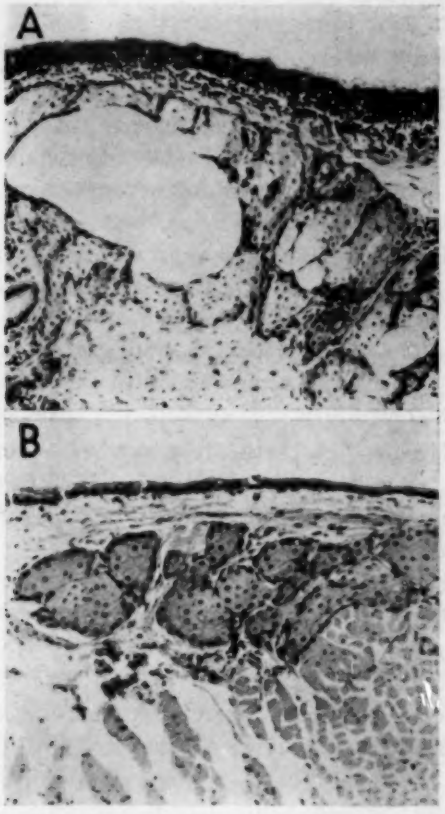


Fig. 2.—*A*, photomicrograph of eyelid from a deficient animal to show the dilated duct of a tarsal gland. The overlying epithelium is thickened and keratinized, with leukocytic infiltration beneath. *B*, photomicrograph (same magnification as in *A*) of eyelid from a control rat to show absence of any dilated duct. The epithelium is lower, and there is no inflammation.

replaced by stratified squamous epithelium. In the subepithelial tissues there were accumulations of leukocytes. No alterations were noted in the intraorbital and extraorbital lacrimal glands at any time. Nor were there any alterations in the harderian glands until the very end of the experiment. At one hundred and forty-two days in some of the deficient

animals there was dilatation of the ducts and acini. The epithelium was flattened but showed no evidence of metaplasia. It is probable that the openings of the ducts were blocked by the inflammatory reaction about them. In the corneas the initial change was an infiltration of the sub-

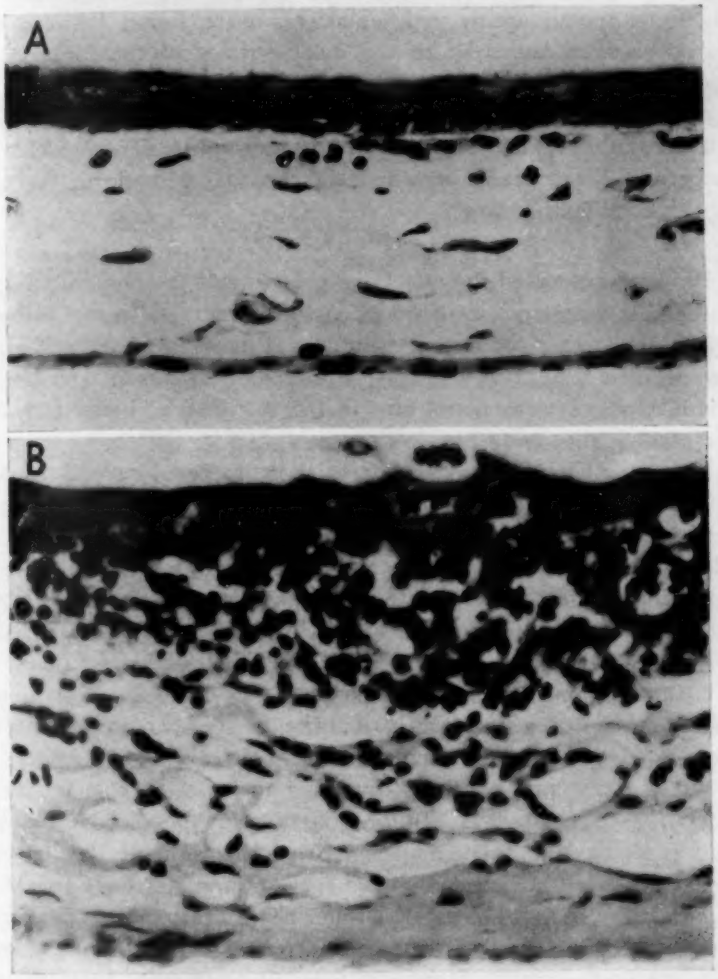


Fig. 3.—*A*, photomicrograph of cornea from a deficient rat to show the earliest change. The epithelium is normal, but beneath, in the substantia propria, there are polymorphonuclear leukocytes. *B*, photomicrograph (same magnification as *A*) of cornea from a deficient rat to show elongation of epithelial cells, intense leukocytic infiltration and presence of capillaries. Note also the increase in the thickness of the cornea in comparison with *A*.

stantia propria beneath the epithelium with polymorphonuclear cells. The epithelial cells at this time appeared unchanged (fig. 3 *A*). Bacteria

could not be stained. As time went on the cellular infiltration of the substantia propria became more and more extensive, and it was accompanied by an ingrowth of capillaries, which made it increase in thickness. The fibers were spread apart. As this was taking place, the corneal epithelium changed its character and became keratinized (fig. 3 *B*). The cells were spread apart, and leukocytes were found between them. Leukocytes were adherent to the inner surface of the cornea. In 1 animal there was an adhesion between the cornea and the iris, and pigment cells were found in the former. Leukocytes were found infiltrating the ciliary body in the later stages. There were large numbers of keratinized fibers and bacteria at the angle of the bulbar and palpebral conjunctivas.

## COMMENT

We have presented the effects on growth and the observations made in a detailed histologic study of the tissues of rats fed a diet containing only 0.002 per cent sodium. Besides the differences in growth between these animals and their controls, whose diet contained 0.66 per cent sodium, changes were noted also in the sex glands, bones and ocular apparatus.

The growth curves are similar to those reported several years ago by Orent-Keiles, Robinson and McCollum.[1] The effect of adding adequate sodium to the diet of the deficient animals is dramatic. The inability of the deficient animals to gain as well as their paired feeding controls was probably due to the difference in water retention and fat synthesis as well as in nitrogen metabolism, which has been studied by Kohlenberg, Black and Forbes [3] and Orent-Keiles and McCollum.[4]

Using the same diet in a previous study, Orent-Keiles, Robinson and McCollum [1] noted a decrease in motility of spermatozoa at about the ninety-sixth day in the animals on the deficient diet. Fertility tests showed that the rats on the sodium-low diet were fertile for at least seventy-five to eighty days. In the present study, histologic examination of testes showed no spermatogenesis from the eightieth day on to the end of the observation period, at the one hundred and forty-second day. Histologically the testes showed the same changes as one sees in inanition. The testes of the animals on the control paired feeding regimen showed active spermatogenesis. However, these animals grew better than the sodium-deficient animals, and this may account for the difference. In other mineral deficiencies we have observed the same picture (Follis, Day and McCollum [5]). We therefore tend to ascribe the change to inanition and not to a specific effect of sodium deficiency.

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3. Kohlenberg, O. J.; Black, A., and Forbes, E. B.: J. Nutrition **13**:97, 1937.
4. Orent-Keiles, E., and McCollum, E. V.: J. Biol. Chem. **133**:75, 1940.
5. Follis, R. H., Jr.; Day, H. G., and McCollum, E. V.: (*a*) J. Nutrition **20**:181, 1940; (*b*) **22**:223, 1941.

In the female animals on this diet, Orent-Keiles, Robinson and McCollum [1] have shown a disturbance in the estrous cycle and virtual cessation of reproductive activity. Vaginal smears showed the presence of cornified cells throughout the entire cycle. Sterility in the rat on diets low in sodium content has also been noted by Miller [6] and St. John.[7] All the female animals in the present study had mature corpora lutea, as well as developing ova, in their ovaries. The uterine epithelium was not atrophic. There was an abundance of cornified cells in the vagina. From the anatomic changes one cannot draw any conclusions as to why these animals tend to be sterile. Factors influencing the implantation and subsequent nutrition of the ovum undoubtedly play a role.

The bones showed virtually complete cessation of growth, such as is seen in inanition from various dietary deficiencies.

In this study we have attempted to gain some idea as to the genesis of the ocular changes. In the lids, swelling was one of the earliest manifestations, and this was considered to be edema by Orent-Keiles, Robinson and McCollum.[1] However, this swelling was due to dilatation of the ducts of the meibomian glands, and it was evident that they were obstructed. This was doubtless caused by a caking of exudate on the lid margins. The epithelial cells lining the inner margin of the lid changed from columnar and goblet cells to stratified keratinized cells. The external and internal lacrimal glands showed no change. In only the later stage of the deficiency were the harderian glands involved. After the animals had been maintained on the diet for twenty weeks, the lumens of the ducts and acini became dilated, owing probably to obstruction because of the inflammation and edema about their openings. The changes in the corneas are most difficult to interpret. Microscopically the initial change was an infiltration beneath the epithelium with polymorphonuclear leukocytes, which indicated some damage to the tissue, though no necrosis or evidence of injury could be made out. Subsequently these increased and there was an invasion by blood vessels. Along with this, the epithelium became keratinized, and the ciliary body became infiltrated with leukocytes also. In 1 instance there was an adhesion of the iris to the cornea. Leukocytes were found in the anterior chamber. Numerous bacteria were found in the conjunctival sac in the later stages.

Ocular changes in rats have been noted in association with deficiencies of other nutritive materials. In vitamin A deficiency Wolbach and Howe [8] noted keratinization of the corneas and of the conjunctival epithelium, while at the same time capillaries invaded the substantia

6. Miller, H. G.: J. Biol. Chem. **70**:759, 1926.
7. St. John, J. L.: J. Biol. Chem. **77**:27, 1928.
8. Wolbach, S. B., and Howe, P. R.: J. Exper. Med. **42**:753, 1925.

propria. Leukocytic infiltration came later. The paraocular glands showed metaplasia of their epithelium too. In rats on a diet deficient in riboflavin Bessey and Wolbach [9] noted vascularization of the cornea, which was followed later by leukocytic infiltration. Follis, Day and McCollum [5b] encountered a somewhat similar picture in rats placed on a diet extremely low in zinc content.

In the corneas described in this paper, the vascular changes seemed to be secondary to an initial injury of the epithelium or of the substantia propria. Capillaries may be made to grow into the normal nonvascular cornea by injecting irritating agents into it, such as tubercle bacilli (Rich and Follis [10]). What the initial change may be is not at all clear. Whether an absence or a diminution of sodium ions in the tears and aqueous humor may play a role is a possibility and should be studied by chemical analyses of these fluids. The possibility that the lysozyme content of the tears may be diminished, thus leading to bacterial infection, must also be considered, though bacteria could not be demonstrated in the initial stages.

SUMMARY

In rats fed a diet adequate in all known respects except that it was extremely low in sodium content, changes were noted in the ocular apparatus, sex glands and bones.

The ducts of the meibomian glands became dilated, and the palpebral conjunctiva became lined by keratinized epithelium. In later stages the harderian glands were dilated. The substantia propria of the cornea became infiltrated with leukocytes; capillaries then invaded it, and the epithelium became transformed to a keratinized type.

Changes in the sex glands and bones were probably due to inanition.

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9. Bessey, O. A., and Wolbach, S. B.: J. Exper. Med. **69**:1, 1939.

10. Rich, A. R., and Follis, R. H., Jr.: Bull. Johns Hopkins Hosp. **66**:106, 1940.

# UREMIA FOLLOWING BLOOD TRANSFUSION

## THE NATURE AND THE SIGNIFICANCE OF THE RENAL CHANGES

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Striking morphologic changes are present in the kidneys in cases of fatal post-transfusion renal insufficiency. That the morphologic changes produce the renal insufficiency is the interpretation favored by the preponderance of evidence. However, this interpretation fails to take into account the occurrence of similar renal changes in conditions that are not associated with renal insufficiency.

The present study is designed to demonstrate the similarity of the changes in the kidneys in persons dying of post-transfusion renal insufficiency to those in deeply jaundiced infants without renal insufficiency and to offer some observations on the significance of these changes. It is arranged in three sections: First, a consideration of the problems posed by the different and conflicting accounts of the renal lesions; second, an attempt to resolve these conflicts and determine the pathologic sequences by a detailed account of the renal changes in 7 cases of delayed transfusion reaction, and, finally, a direct comparison of these changes with those occurring in cases of deep jaundice.

### CLINICAL AND PATHOLOGIC CHARACTERISTICS

Bordley[1] has pointed out that patients with renal insufficiency following blood transfusion generally have a highly characteristic clinical course. During or immediately after the transfusion there is a febrile reaction, followed frequently by hemoglobinuria, occasionally by jaundice and invariably by oliguria. The oliguria persists and the nonprotein nitrogen rises, but symptomatic improvement occurs during the next several days. After this interval, agitation or drowsiness sets in, followed by evidence of uremia. Death with convulsions and coma occurs in the

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1. Bordley, J.: Arch. Int. Med. **47**:288, 1931.

majority of cases, but at any time there may be a sudden diuresis with recovery. In a review of the observations at autopsy in the cases reported up to 1930, Bordley described a renal change of unusual type.

> . . . It is characterized chiefly by edema and cellular infiltration of the interstitial tissue, dilatation of the intracapsular spaces of the glomeruli, dilatation of the convoluted tubules, advanced necrotic changes in the tubular epithelium, especially that of the collecting tubules, with deposition of pigmentd droplets in many of the cells and finally, the appearance in the tubular lumina of leukocytes, large phagocytic wandering cells, desquamated epithelium and peculiar pigmented globules of various sizes. Studies of the droplets in the epithelial cells and of the larger pigmented masses in the lumina of the tubules invite the suggestion that they are hemoglobin products of some sort. The droplets seen near the glomerular extremity of the tubule possess the staining characteristics of the hemoglobin that is contained in adjacent erythrocytes, but those which occur further down toward the renal pelvis have lost their eosinophilic and fuchsinophilic qualities and show increasing amounts of brownish pigmentation. Except for the occurrence of this pigment, the changes in the kidney are much like those found after poisoning with mercuric chloride.

Renal changes conforming in general to this description are found in the majority of patients with the condition in question and in rabbits and dogs with the type of renal insufficiency produced by intravenous administration of hemoglobin when the urine is acid.[2] Although some authors have considered that blockage of tubules by casts causes the renal insufficiency,[3] others that epithelial degeneration is the cause [4] and still others that each of these factors plays an independent role,[2b] they find common ground for agreement in considering the renal changes as the cause of the insufficiency. On the other hand, Kimmelstiel [5] did not find the evidence sufficient to support this. Necrosis of epithelial cells may be absent, and casts may be so small and few that mechanical blockage is out of the question. He pointed out the occurrence of edema and cellular infiltration of the interstitial tissue, often without a satisfactory morphologic explanation. This, he held, is a morphologic manifestation of hyperergy; the shutdown of the kidneys he considered a functional manifestation of the same reaction. Since hyperergy may cause either of these manifestations, he found it not surprising that they often occur together.

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2. (*a*) Baker, S. L., and Dodds, E. C.: Brit. J. Exper. Path. **6**:247, 1925. (*b*) De Gowin, E. L.; Warner, E. D., and Randall, W. L.: Arch. Int. Med. **61**:609, 1938.

3. Ponfik: Virchows Arch. f. path. Anat. **62**:273, 1875. Levy, L.: Deutsches Arch. f. klin. Med. **81**:359, 1904. Yorke, W., and Nauss, R. W.: Ann. Trop. Med. & Parasitol. **5**:287, 1911. Bell, E. T.: Am. J. Path. **13**:497, 1937. Baker and Dodds.[2a]

4. (*a*) Goormaghtigh, N.: Arch. méd. belges **72**:611, 1918. (*b*) Goldring, W., and Graef, I.: Arch. Int. Med. **58**:825, 1936.

5. Kimmelstiel, P.: Am. J. Path. **14**:737, 1938.

This outline of the chief differences among those who have studied the kidneys in this condition serves to indicate why it is necessary to reexamine the morphologic sequences in post-transfusion renal insufficiency in order to obtain a basis for comparison with other conditions.

The following series of cases affords material for the determination of the lesion that is *progressive* and many of its morphologic sequences. These cases will serve to demonstrate that the necrosis of epithelial cells and the inflammatory reaction in the interstitial tissue form a single process, that the casts do not, by erosion, cause this necrosis of epithelial cells and, finally, that the blockage of tubules by casts is insufficient to serve either as the cause of the oliguria or as the primary injury leading to the final morphologic picture. With the essential morphologic characteristics of the kidneys in the delayed transfusion reaction determined, it will be possible to compare these changes with those occurring in the kidneys of deeply jaundiced infants.[6]

## REPORT OF CASES

Autopsy material for microscopic examination was obtained through the assistance of Dr. E. Granville Crabtree in 6 cases of uremia following transfusion reaction and in 1 case in which the patient died three and a half hours after an immediate reaction. Although this patient died without clinical evidence of renal insufficiency, the case is included to show the appearance of the kidney shortly after an immediate transfusion reaction. In case 2 there was only a single section of kidney, fixed in a 4 per cent solution of formaldehyde and stained with hematoxylin and eosin. In cases 1 and 3 there were sections of kidneys fixed in Zenker's fluid and stained with hematoxylin and eosin and Mallory's aniline blue. In cases 4, 5, 6 and 7, kidney material fixed in a 4 per cent solution of formaldehyde and in Zenker's fluid was available. The sections of material fixed in Zenker's solution were stained with hematoxylin and eosin, Mallory's aniline blue and Mallory's phosphotungstic acid–hematoxylin. The formaldehyde-fixed sections were prepared with Turnbull's blue iron stain, von Kossa's stain for calcium and scarlet red–hematoxylin stain.

Case 1 (Dr. Frederick Parker, Boston City Hospital).—A woman aged 37 was given a transfusion for postpartum hemorrhage and died within three hours.

*Microscopic Observations on the Kidneys.*—The general structure of the kidneys was well preserved. There was no engorgement, edema, cellular infiltration or scarring. The epithelial cells of the proximal convoluted tubules were swollen, granular and frayed; their nuclei were pale or pyknotic, seldom normal. The lumens of these tubules were filled with pale acidophilic granular debris, which at times assumed the outlines of circular reticulum. The glomerular tufts almost completely filled the capsular spaces. The epithelium of the glomeruli, particularly that covering the tufts, showed a similar cloudy swelling, and the slitlike capsular spaces contained circular reticulum. There was no increase of cells. The basement membranes were thin. However, the cloudy swelling of the tuft epithelium and the fading of many red blood cells gave the tufts a swollen, bloodless appearance. Occasional tubules of Henle contained pale acidophilic debris, but most of them

6. Ayer, D.: Arch. Path. **30**:26, 1940.

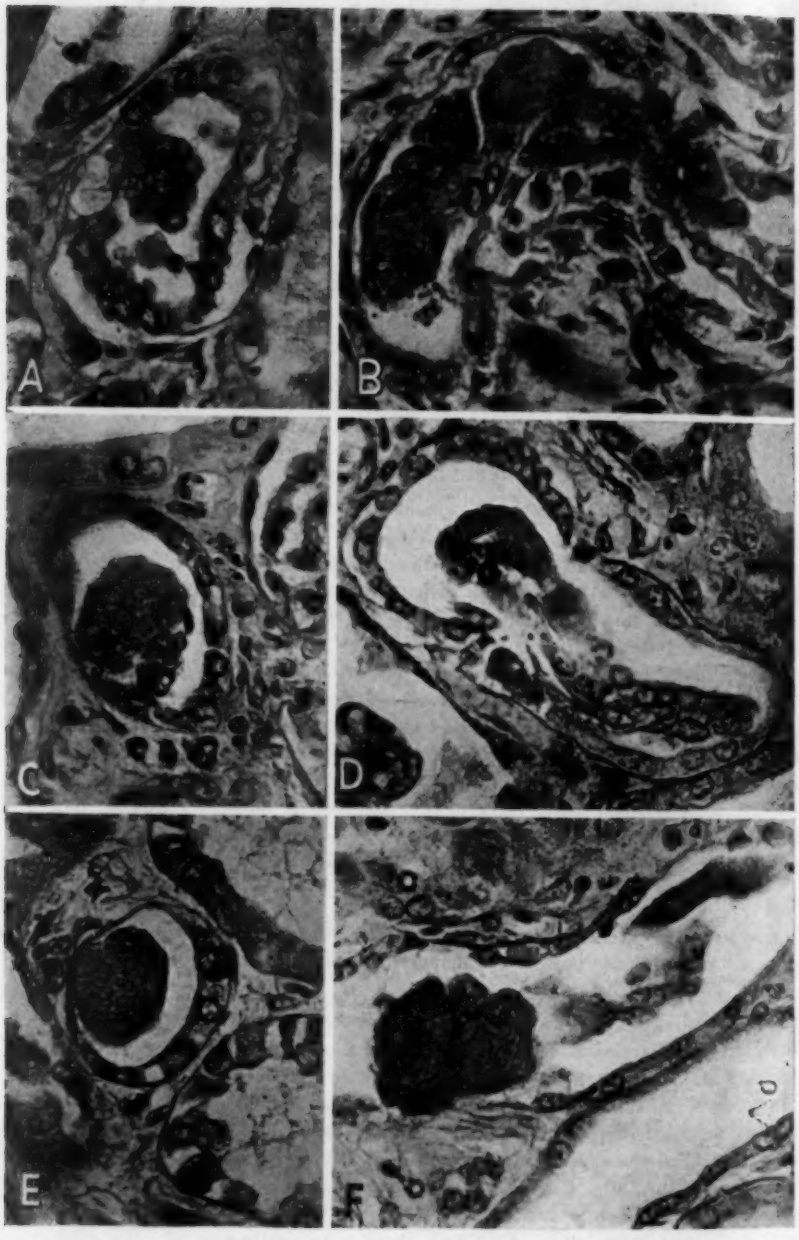


Figure 1

*(See legend on opposite page)*

were empty. The lining cells of the ascending and descending limbs occasionally contained small droplets of pale brown pigment.

In the lumens of distal convoluted tubules, collecting ducts and collecting tubules of the medulla there was material that differed completely from the pale, acidophilic, finely granular material that was present in the lumens of the proximal convoluted tubules and some of Henle's tubules. This material occurring distally was usually coarsely granular and was either brick red or brown. In all locations the brick red material predominated. Rarely the material was compacted into loose casts that filled but did not distend the lumens. The material was most abundant in the collecting ducts at the junction of the cortex and the medulla. A smaller amount was present in the large collecting tubules of the medulla, and a still smaller amount in the lumens of the distal convoluted tubules. There were no leukocytes mixed with this material. The epithelial cells of these distal tubules contained no brick red material within the cytoplasm and showed no degenerative changes. Occasional otherwise normal cells of the large collecting tubules of the medulla contained droplets of pale brown pigment, but the cells of the distal convoluted tubules and the collecting ducts did not contain even this material. There was moderate arteriosclerosis, as well as slight arteriolosclerosis. There were no thrombi.

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EXPLANATION OF FIGURE 1

*A*, distal convoluted tubule with a projecting bud of intact epithelial cells. In one of the lining cells beneath the bud there is a small irregular mass of the brick red material. Case 5; hematoxylin and eosin stain; × 472.

*B*, distal convoluted tubule containing epithelial cells filled with brick red and brown material. One mass of cells filled with this material is continuous with the cytoplasm of the cells lining the tubule. The other masses of cells are apparently detached. There is also edema of the surrounding interstitial tissue. Case 5; hematoxylin and eosin stain; × 472.

*C*, segment of a distal convoluted tubule applied to the vascular pole of a glomerulus. The epithelial bud contains viable cells only in the lower part on one side. In the greater part of the bud, the cells are granular and indefinite in outline, with faded nuclei. There is faint brownish discoloration of these cells. Case 5; hematoxylin and eosin stain; × 472.

*D*, large sheet of cells projecting into the lumen of the tubule in the center. Below and to the right the cells forming the sheet stain normally. Above and to the left there are several small dark masses which stained deeply acidophilic. In the lining epithelial cells beneath the large sheet of cells there is a large mass of faintly pigmented acidophilic material. Case 5; hematoxylin and eosin stain; × 472.

*E*, epithelial bud, attached to the basement membrane of a tubule (in the center), from which cell outlines and nuclei have disappeared. At the upper pole the dark oval mass and the several dark points are made up of brick red material; the remainder of the granular material is tinted with brown. About the free margin is a narrow rim of basophilic cytoplasm. Case 5; hematoxylin and eosin stain; × 472.

*F*, group of unattached cells swollen with brown granular material alone. The nuclei have faded out. About the periphery of the cells is a narrow rim of basophilic cytoplasm. Case 5; hematoxylin and eosin stain; × 472.

CASE 2 (Dr. John Fallon, Worcester, Mass.).—A woman aged 38 years was given a transfusion for hemorrhage following hysterectomy for leiomyoma of the uterus. Death occurred within three days.

*Microscopic Observations on the Kidneys.*—The blood vessels were slightly engorged, but there was no edema, leukocytic infiltration or scarring of the interstitial tissue. A single interlobular vein contained a small mural fibrinous thrombus. The glomerular epithelium was very slightly swollen, but there was no increase in the number of cells. Circular reticulum appeared in the capsular spaces, but these spaces were not dilated. The basement membranes were thin. The proximal convoluted tubules were all slightly dilated and filled with acidophilic debris and circular reticulum. The epithelial cells lining these tubules were slightly swollen and frayed, but the nuclei were well preserved. Henle's tubules were empty or contained only minute amounts of pale acidophilic debris. Golden brown pigment was present in nearly all the cells of the descending limbs and in occasional ones of the ascending limbs of Henle's loops, but these cells were otherwise well preserved. The distal convoluted tubules and collecting ducts contained large amounts of brick red debris mixed with occasional large and small droplets of pale yellow or brown pigment. This material was occasionally compacted into casts, which slightly distended the tubules they occupied. Rarely, groups of epithelial cells enveloped masses of this brick red material mixed with occasional brown droplets (fig. 1 *B*). There was no instance of a breakdown of the cells lining these tubules to give a reaction in the surrounding tissue or to call forth leukocytic infiltration.

The collecting tubules of the medulla contained a smaller amount of material, very loosely arranged. In this location the amount of brownish material was greater than the amount of brick red material. The cells of these tubules contained no droplets of any kind. There was very slight evidence of arteriosclerosis and arteriolosclerosis.

CASE 3 (Dr. James C. McCann, Worcester, Mass.).—A woman aged 39 years received a transfusion for hemorrhage following incomplete abortion. Death ensued within six days.

*Microscopic Observations on the Kidneys.*—The two most striking features were the numerous small casts in the tubules and the heavy leukocytic infiltration of the interstitial tissue in a broad zone of the medulla, immediately beneath the cortex. In the cortex, edema of the interstitial tissue was the prominent finding. Under higher magnification it could be readily established that the casts were not confined to the medulla but were, in fact, confined to the collecting tubules in the cortex and the medulla and the distal convoluted tubules. This material, referred to as casts, varied greatly in color, texture and density. In the majority of the tubules it formed finely granular, brick red casts that filled and slightly distended the tubules. In other tubules the finely granular material was more loosely packed, smaller in amount and had a reddish brown hue. In still others the casts were made up of slightly larger droplets with a yellow or greenish yellow color. Some casts were composed of mixtures of these types of material. There was no significant difference in the total amount of cast material at various levels nor any clearcut difference in the relative amounts of the material of various colors at different levels. For example, it was possible to find three neighboring segments of distal convoluted tubule, one containing only brick red material, the second containing only pale green material and the third containing equal amounts of

the two. A similar distribution of material was found in the large collecting tubules of the medulla. However, in general, there was more brick red material in the distal convoluted tubules and collecting ducts, and more green or yellow material in the large collecting tubules of the medulla. In the distal convoluted tubules and collecting ducts epithelial cells contained masses of brick red material. Rarely, only a single very small droplet appeared in an otherwise normal cell that differed in no other way from its neighbors. At times a single cell was distended to form a thin rim of cytoplasm about a rounded mass; at other times the cytoplasm of a multinucleated cell formed a continuous ring about a larger mass of this material. Some of the clumps of epithelial cells and cast material were undergoing degeneration, as evidenced by leukocytic infiltration. When these cast-filled cells were attached to the basement membrane, this infiltration was accompanied by cellular infiltration and edema of the surrounding stroma. These tubules with degeneration of the lining epithelium were more numerous and closer together in the subcortical portion of the medulla, and the cellular infiltration about them gave the appearance of heavy infiltration of the interstitial tissue in this part of the kidney. Such degenerative changes were fewer and milder in the collecting ducts and distal convoluted tubules in the cortex. About them there was less cellular infiltration with consequent prominence of the edema of the surrounding interstitial tissue. Rare mitotic figures were observed in the epithelial cells of the distal convoluted tubules and the collecting ducts. Although the lumens of the large collecting tubules in the medulla often contained numerous polymorphonuclear leukocytes mixed with the cast material, the lining cells contained no red or brown droplets, and, even more important, there was no cellular infiltration of the interstitial tissue about these large collecting tubules of the medulla. The cells of Henle's loops contained clumps of golden brown pigment, but these cells exhibited no evidence of proliferation or degeneration, and the lumens contained only rare bits of pale acidophilic debris. The proximal convoluted tubules were slightly dilated and filled with circular reticulum. The epithelium was low but well preserved. The glomerular capsules were slightly dilated and contained small amounts of circular reticulum. There was no increase of cells, swelling of the epithelial cells or thickening of the basement membranes of the tufts. The larger arteries showed slight arteriosclerosis, but the smaller arteries and arterioles were normal. There were no thrombi.

CASE 4 (Dr. Tracy Mallory, Massachusetts General Hospital).—A woman aged 46 years died seven days after hysterectomy followed by a transfusion for mild secondary anemia, caused by vaginal bleeding of two months' duration.

*Microscopic Observations on the Kidneys.*—These kidneys differed from those in case 3 principally in the smaller number of casts. These were composed of material of the same three types, i. e., fine brick red granules, fine yellowish brown granules and larger yellowish green droplets. This material was present in small clumps that did not distend the tubules. It was confined to the distal convoluted tubules, collecting ducts and collecting tubules and occurred in approximately the same proportions in each of these segments. Epithelial cells containing this material were frequently encountered, and degeneration of these cells with leukocytic infiltration was present; but this intracellular material, with or without degeneration of epithelial cells, occurred only in the distal convoluted tubules and collecting ducts. The cells in and about the degenerating tubules were partly polymorphonuclear leukocytes and partly a mixture of monocytes, lymphocytes

and fibroblasts with occasional eosinophils and plasma cells. These cells were more numerous in and about the degenerating distal convoluted tubules and collecting ducts of the cortex. In the distal convoluted tubules and collecting ducts, rare mitotic figures were found. Fat droplets were present in the epithelium of the distal convoluted tubules and collecting ducts, but there was no iron or calcium. Although the lumens of the large collecting tubules of the medulla contained polymorphonuclear leukocytes mixed with loosely scattered bits of cast material, the cells lining these tubules showed no degenerative changes, and the interstitial tissue about them was free from inflammatory reaction. The cells lining Henle's tubules contained round clumps of iron-free yellow granules and fat droplets, but they exhibited no degenerative changes and the lumens contained only small bits of pale acidophilic debris. The proximal convoluted tubules were slightly dilated and filled with circular reticulum. The epithelial cells were low but otherwise normal. The glomerular tufts showed a slight increase in cells. The capsular spaces were slightly dilated and contained circular reticulum. A single vein contained a mural thrombus of fibrin. There was slight arteriosclerosis, but the arterioles were normal.

Case 5 (Dr. Arthur T. Hertig, Boston Lying-In Hospital and the Free Hospital for Women).—A woman aged 22 years was given a transfusion for shock caused by inversion of the uterus after delivery. Death occurred within eight days.

*Microscopic Observations on the Kidneys.*—Smaller bits of cast material were present in two thirds of the distal convoluted tubules and collecting ducts. Red and brown material was present at all levels, with a slight predominance of the red. For the most part this material was surrounded by the cytoplasm of epithelial cells that were either free in the lumen or, more rarely, attached to the basement membrane. Rare mitotic figures were present in the distal convoluted tubules and collecting ducts. Edema of the interstitial tissue was present only about the distal convoluted tubules and collecting ducts, which often showed evidence of epithelial degeneration. Where there was leukocytic infiltration of these injured epithelial cells, a similar infiltration was usually present in the interstitial tissue about them. Where a tubule was disrupted and leukocytes were few, there was usually slight edema, but occasionally this was absent. Occasional buds of intact epithelial cells were found (fig. 1*A*). Fat droplets were present in the cells and cast material of the distal convoluted tubules and collecting ducts and in the cells of Henle's tubules. The cells of Henle's tubules that contained fat droplets also contained clumps of pale brown granules, but these cells were otherwise well preserved, and the lumens contained only finely granular acidophilic material. The large collecting tubules of the pyramids were usually empty, containing only rare red and brown globules in the lumens. Occasionally, polymorphonuclear leukocytes were mixed with this material, but the cells lining these large tubules showed no pigmentation or degeneration, and the surrounding interstitial tissue was, for the most part, free from inflammatory reaction. The proximal convoluted tubules were slightly dilated and filled with circular reticulum. The cells were slightly swollen and their free ends frayed, but their nuclei were normal. The glomerular capsules were very slightly dilated and contained small bits of circular reticulum. The capsular epithelium was slightly swollen and frayed. The tufts were well filled with blood; the basement membranes were thin. There were three observations of a close application of large thin-walled veins to degenerating tubules. In each instance the leukocytes that lay about the degenerating tubules extended into the wall of the vein, and the intima over the area of infiltration was covered

with a small amount of fibrin. Other small mural thrombi were not associated with demonstrable leukocytic infiltration of the vessel wall. The arteries and arterioles were normal.

CASE 6 (Dr. Tracy Mallory, Massachusetts General Hospital).—A woman aged 50 years was given a transfusion for septic sore throat. Death followed in ten days.

*Microscopic Observations on the Kidneys.*—Casts were rare and small but were most numerous in the distal convoluted tubules. They were present only in the distal convoluted tubules, collecting ducts and collecting tubules. The cast material was predominantly the finely granular brown variety, but a small amount of the brick red type was present. There were occasional instances of the inclusion of this material within epithelial cells, and rare instances of degeneration of these cells with leukocytic infiltration in and about the tubules containing them. These degenerating cells occurred principally in the distal convoluted tubules, rarely in the collecting ducts and never in the large collecting tubules of the pyramids. Small fat droplets were present in these degenerating cells and in the intact pigment-containing cells of Henle's loops, but there was no iron or calcium. There was slight evidence of edema about some of the cast-containing distal convoluted tubules and collecting ducts. No mitotic figures were encountered. Although the lumens of the collecting tubules of the pyramids contained a small amount of cast material and a few polymorphonuclear leukocytes, there was no change in the lining epithelium or in the surrounding interstitial tissue. The cells of Henle's tubules contained clumps of pale brown granules, but there were no degenerative changes, and the lumens were empty. The proximal convoluted tubules were very slightly dilated and contained very small amounts of circular reticulum. The lining cells were slightly swollen and irregular but seldom frayed. The glomerular capsules were slightly dilated, the epithelial cells slightly swollen, and small amounts of circular reticulum were present. The basement membranes were thin. Rare glomeruli were completely hyalinized, but no intermediate sequences were present. Many larger arteries showed moderate arteriosclerosis. The smaller arteries were normal. There were no thrombi.

CASE 7 (Dr. Arthur T. Hertig).—A woman aged 35 years was given a transfusion because of bleeding from a fibroid uterus. Decapsulation and biopsy were done three days after the transfusion. Death occurred within ten days.

*Biopsy of the Kidneys.*—The specimen consisted entirely of the outer cortex. Most of the distal convoluted tubules and collecting ducts were filled with brick red material, disposed in small globules and tiny granules. Rarely bits of this material had a faintly brownish cast, but there was no regular arrangement of this discoloration. Occasionally the tubules were greatly distended with this material, but an equal number of segments of the distal convoluted tubules and collecting ducts were empty. Changes occurred in the epithelial cells of these two segments whether the tubules were filled or empty. The simplest change was a slight piling up of the cells to form a low bud. Larger cellular buds were more rarely encountered. In other locations the cells of such buds contained tiny bits of the brick red material. Larger buds consisted of a central core of brick red material with a peripheral covering of flattened epithelial cells. Many of the buds, irrespective of type or contents, were infiltrated by small numbers of polymorphonuclear leukocytes. In the interstitial tissue about many of these distal tubules lay small accumulations of polymorphonuclear leukocytes and a smaller number of monocytes and lymphocytes. Even when infiltrating cells were absent, the

interstitial tissue about the distal convoluted tubules and collecting ducts was edematous. This edema occurred only about the distal convoluted tubules and the collecting ducts.

The proximal portions of the nephron contained no brick red material. The epithelial cells gave no evidence of necrosis. The glomerular spaces and the lumens of the proximal convoluted tubules were slightly dilated and filled with circular reticulum. This was particularly marked in the proximal convoluted tubules, where the lining cells were reduced in height by the exaggerated production of circular reticulum. Henle's tubules were empty, and their lining cells were well preserved and free from pigment. There were occasional hyalinized glomeruli, but no active degenerative or inflammatory process was evident. The arteries and veins were normal. There were no thrombi.

*Postmortem Microscopic Observations on the Kidneys.*—Progression of the lesions in the distal convoluted tubules and collecting ducts to the stage of advanced necrosis and early repair formed the most striking feature of the kidneys post mortem. These lesions were not present in the pyramids; they occurred only in the outer part of the medulla and in the cortex. At first glance they appeared to be simply large foci of heavy cellular infiltration, but on closer inspection, in many instances the remains of a necrotic tubule were identified in the center of the cellular focus. In some instances this was unmistakable: The lining cells were still present, some of them containing brick red material. Polymorphonuclear leukocytes filled the lumen and lay among the epithelial cells. About the tubules were large numbers of polymorphonuclear leukocytes and smaller numbers of monocytes and lymphocytes. Often a small number of fibroblasts were present. In other instances a small irregular mass of brick red material lay in the the center surrounded by leukocytes and fibroblasts. In such areas there were no epitheial cells to permit positive identification of a tubule. In still other foci even the presumptive evidence of brick red material was lacking. Earlier lesions, such as small cellular buds and bits of brick red material surrounded by a rim of cells, were present, but they were extremely rare. Likewise, it was immediately evident that although the preserved distal convoluted tubules and collecting ducts contained some brick red material, these tubules contained but a small fraction of the amount that was present at the time of the biopsy. The largest amount of cast material was found in the large collecting tubules of the medulla. Approximately one third of these tubules were slightly distended with irregular fragments of cast material mixed with leukocytes. Despite the presence of leukocytes in the lumens of these tubules, there was no evidence of necrosis of the lining epithelial cells, nor was there any inflammatory reaction in the surrounding interstitial tissue. The color of the cast material in the distal convoluted tubules, collecting ducts and collecting tubules was uniformly brick red with a slight brownish discoloration. There was no increase in the depth of the brownish discoloration of the material in the more distal tubules. The proximal convoluted tubules and Henle's tubules contained a small amount of circular reticulum, but the lining cells were higher, making these tubules appear less dilated than those in the biopsy sections. Occasionally, the cells lining Henle's loops contained pure golden brown droplets, but the lumens of these tubules were empty. The glomerular tufts almost completely filled the capsular spaces and were slightly more cellular than normal. The arteries and veins were normal. The surface of the kidney was covered by a thin but regular fibrous envelope. The cells were plump with prominent nuclei. There were no infiltrating cells beneath this capsule.

SPECIAL CONSIDERATIONS

The length of time between the blood transfusion and death in each case was considered most likely to be a fair measure of the duration of the acute process in the kidney. When the cases were arranged in order of increasing duration, it became apparent that the variations in the cast material and in the venous thrombi did not take place in accordance with any time sequence; that the changes in the epithelium of the proximal portion of the nephron were more severe in the earlier cases and less marked in the cases of longer duration, and that the changes in the epithelium of distal tubules and in the interstitial tissue were absent early, appeared later and increased in severity. Moreover, there was a definite relation between the character of these epithelial changes and the edema and leukocytic infiltration of the interstitial tissue.

In case 2, with a kidney shut-down for three days, brick red and brownish material appeared ***within*** the cytoplasm of the epithelial cells lining the distal convoluted tubules and collecting ducts. In spite of the great swelling occasioned by the presence of this material, these cells appeared to be viable, judging by their well preserved nuclei and by the absence of infiltrating leukocytes. In case 1 none of this intracellular material was found, but in the biopsy in case 7 and in case 2, at three days, and in the cases of six, seven, eight and ten days' duration, the intracellular material was present, and many cells containing this material were infiltrated by polymorphonuclear leukocytes. Only when this evidence of epithelial necrosis was present were there edema and leukocytic infiltration of the interstitial tissue or were leukocytes present in the lumens of occasional collecting tubules.

A more detailed account of the changes in the various anatomic subdivisions of these kidneys will serve to strengthen the impression that the changes which determine the complicated morphologic picture are those in the epithelium of the distal convoluted tubules and collecting ducts.

*Distal Tubules.*—In the search for the early lesions that led to the leukocytic infiltration, attention was directed to the tubules distal to those of Henle, for it was only in the lumens of the distal convoluted tubules, collecting ducts and collecting tubules that polymorphonuclear leukocytes and macrophages were found within the nephron. The search was further narrowed by the location of the epithelial cells that contained brick red or pigmented segregated material. Such cells were found still attached to the basement membrane only in the distal convoluted tubules and collecting ducts. Therefore, it was in this portion of the distal system that the simplest changes were sought.

The simplest change was a piling up of epithelial cells, actually forming globular buds in some instances (fig. 1 *A*). Rarely the cells

forming such a bud and those beneath it contained no brick red or brown-stained segregated material, but more commonly irregular small bits of such material could be found in the cells of the bud, in the cells beneath it or in both.

The further development of the change that began by the formation of these buds took one of three different forms. The commonest change was the distention of the cells by a larger amount of the segregated material (fig. 1 *B*). Less commonly there was an increase in the number of cells, forming a larger mass projecting into the lumen of the tubules (fig. 1 *C*). Rarely a sheet of these cells, still well preserved, lay free in the lumen. Cells in these sheets were at times filled with segregated material; at other times they contained none.

The next change in the order of severity was the appearance of the first positive evidence of cell degeneration. This was the fading of the nuclei in the epithelial cells with faint brownish discoloration of the vaguely outlined former site of the nucleus. This change could be found involving a small proportion of the cells in a single bud (fig. 1 *D*) or all of the cells of the bud (fig. 1 *E*). Usually the cytoplasm of the cells with faded nuclei stained more deeply basophilic; particularly was this true of the sheets of detached cells, whether the cells contained segregated material (fig. 1 *B*) or not (fig. 1 *F*).

Next in the order of increasing severity of this series of changes was the appearance of the first unmistakable evidence of necrosis of epithelial cells—the invasion of individual cells by polymorphonuclear leukocytes. Such infiltration was found in small and large buds, with and without segregated material (fig. 2 *A* and *B*). Often the lumen nearby contained no casts or leukocytes. This gave support to the impression that the leukocytic infiltrations of these epithelial cells was not secondary to changes in the contents of the tubules or to mechanical injury to these cells by casts, but was, in reality, a change that preceded the filling of the distal tubules with leukocytes and that occurred where no casts were present. Between the early infiltration of degenerating cells by leukocytes and the final disruption of the tubule (fig. 3 *A*) and surrounding infiltration various stages occurred (figs. 2 *C* and *D*).

It is important to emphasize that none of the earlier, simpler changes were found in the large collecting tubules of the medulla. Often these tubules were distended with a mixture of cast fragments, desquamated cells and large numbers of leukocytes, while the lining epithelium was intact and morphologically normal. In other such distended tubules, the lining epithelium was absent, but there was no inflammatory cell infiltration about the tubule. Rarely, however, a small number of inflammatory cells were present about a collecting tubule distended with the mixture of cast fragments and leukocytes. However, the conclusion

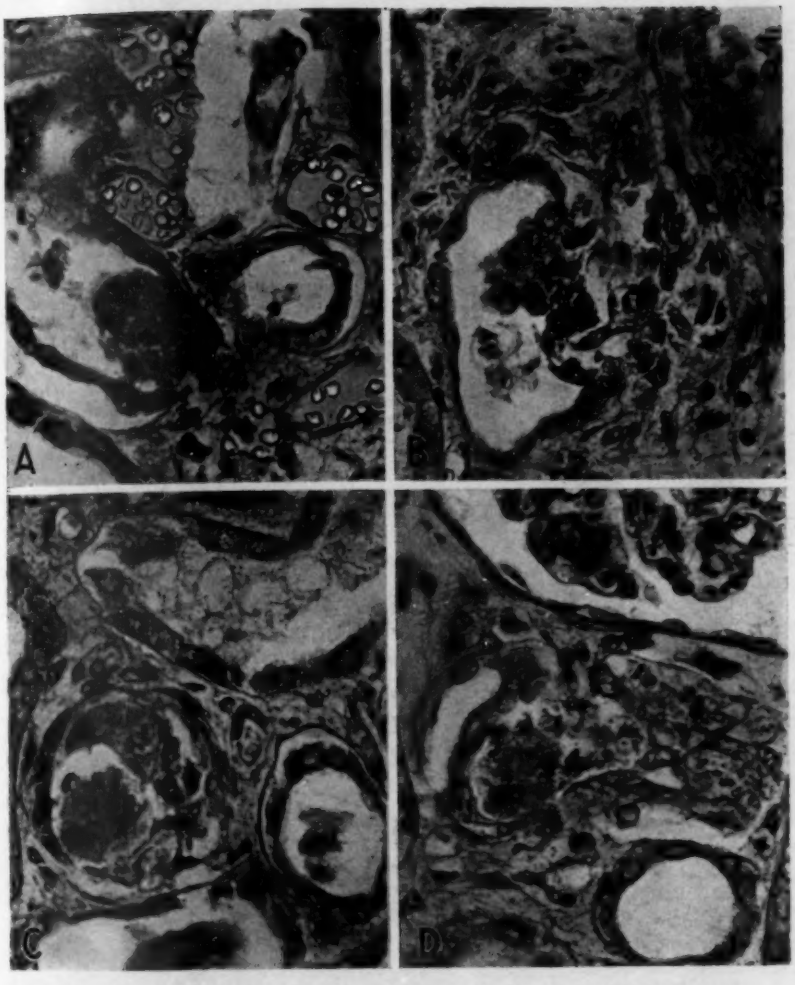


Fig. 2.—*A*, epithelial bud undergoing necrosis. The nuclei of three polymorphonuclear leukocytes are seen in the bud. To the right in the base is a group of brick red droplets, seen as dark spots. Otherwise the matrix of the bud is made up of faintly brownish granular material. Case 5; hematoxylin and eosin stain; × 472.

*B*, very small bud, with only about four cells discernible, projecting into the lumen of a tubule (center). In the midst of these cells a single dark nucleus of a polymorphonuclear leukocyte is visible. About the tubule there is edema of the interstitial tissue, with prominence of the nuclei of the endothelial and connective tissue cells. Case 5; hematoxylin and eosin stain; × 472.

*C*, tubule containing several cells filled with the red and brown material. These swollen cells are attached to one another and, in the lower portion, are still attached to the basement membrane. Several nuclei are preserved, but there is at least one infiltrating polymorphonuclear leukocyte. Case 5; hematoxylin and eosin stain; × 472.

*D*, cells of a distal convoluted tubule containing large amounts of brick red material and infiltrated with numerous polymorphonuclear leukocytes. Kidney in case 5; hematoxylin and eosin stain; × 472.

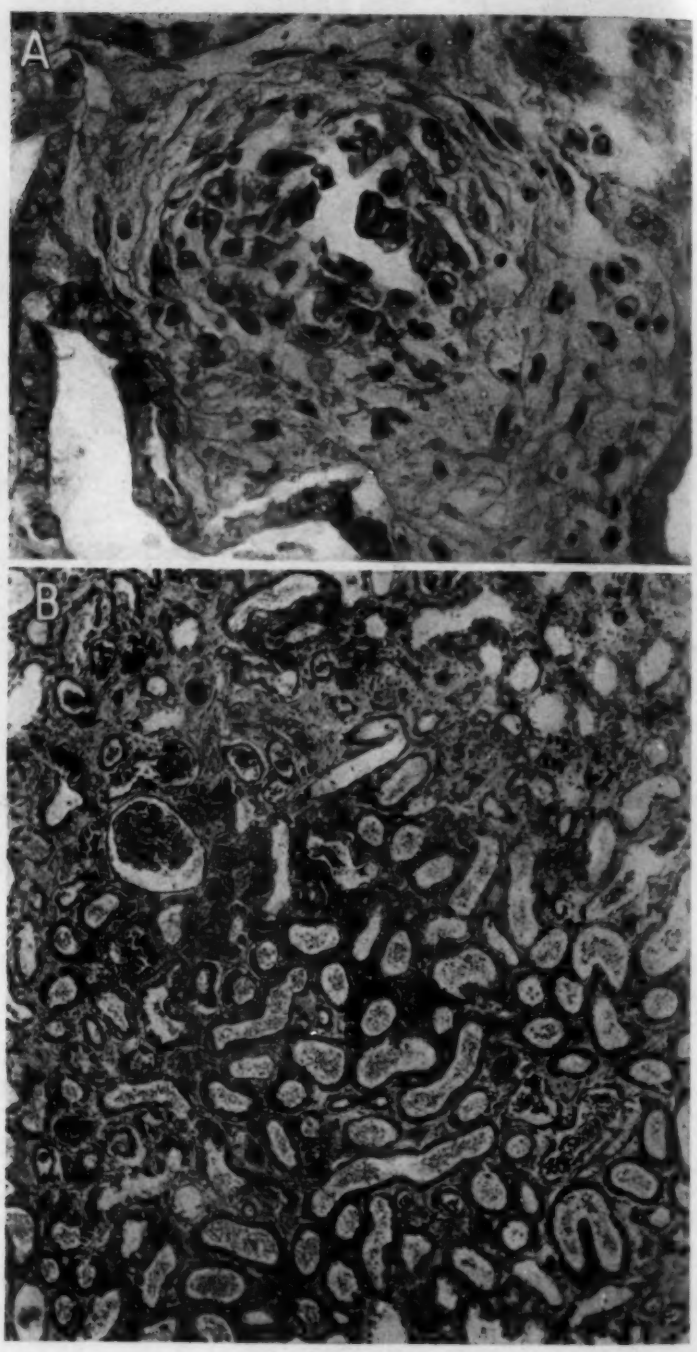


Figure 3

*(See legend on opposite page)*

seemed reasonable that this reaction was produced not by sequences of epithelial cell degeneration as in the distal convoluted tubules and collecting ducts but by the inflammatory material from above that had reached these lower tubules.

*Casts and Intracellular Material.*—The chemical nature of the material forming the casts in human kidneys with post-transfusion renal insufficiency has not been determined. The material has been regarded as hemoglobin and products of the breakdown of hemoglobin, mixed in various proportions.[3] Although there was strong presumptive evidence that the red and variously pigmented material in the tubules was derived from hemoglobin, there was no method whereby it could be positively identified in fixed material. However, comparison of the material in these cases with that described in other cases showed them to be similar in ordinary stains. The material was disposed in droplets or globules of various sizes, the largest with a diameter slightly greater than that of an erythrocyte, the smallest of a size less than that of a nucleolus. The globules were seldom perfectly round; they were usually ovoid or irregular in contour. Their color (in the hematoxylin and eosin stain after fixation in formaldehyde or Zenker solution) varied from the brick red of the usual erythrocyte through shades of yellow or pale green to a deep brown. Bordley [1] and Lindau [7] reported that the droplets in the proximal portion of the tubules were deeply eosinophilic while those in the tubules toward the pelvis progressively lost their eosinophilic qualities and showed increasing amounts of brownish or greenish pigmentation. This gradation was insignificant or absent in the present series of cases. In each of the cases both colors were present. In cases 1, 5 and 7 the red material predominated in each level. In cases 2, 3 and 4 the two kinds of material were present in about equal proportions at each level.

Certain authors have suggested that the droplets in the epithelial cells were also hemoglobin products of some sort [8] and showed the same

7. Lindau, A.: Acta path. et microbiol. Scandinav. **5**:382, 1928.
8. Yorke, W.: Ann. Trop. Med. & Parasitol. **5**:401, 1911.

EXPLANATION OF FIGURE 3.

*A*, complete disruption of a tubule with edema and cellular infiltration of the surrounding interstitial tissue. Case 5; hematoxylin and eosin stain; × 500.

*B*, kidney in case 5; × 50; phosphotungstic acid-hematoxylin stain. Note the distribution of the edema of the interstitial tissue. In the center of the field, the tubules with thick, deeply staining lining cells are proximal convoluted tubules. These lie pressed against one another. The smaller tubules, with lower, paler lining cells and with dark material in the lumens, are for the most part distal convoluted and collecting tubules. They are separated from one another by edematous interstitial tissue.

change of color from red in the proximal portion to brown in the distal portion of the tubules as was found in the material in the lumens of the tubules.[1] In the present series this gradation of color was not present. The distribution was, in fact, totally different. Red material and brown pigment in varying proportions occurred in the epithelial cells of the distal convoluted tubules and collecting ducts. Proximal to these segments (in Henle's loops) and distal to them (in the collecting tubules) the epithelial cells contained no red material, but in some cases there were brown droplets in these cells.

The importance of this distribution was shown by the fact that necrosis of epithelial cells was found only in the segments of tubules to which the *intracellular* red material was confined. Furthermore, the innocuous nature of the brown pigment was shown by the complete absence of necrosis of the epithelial cells in those segments in which the red material was constantly absent and the brown pigment often present. A further difference between the brick red material and the pure brown pigment, such as that which occurred in Henle's loops, was brought out by the reaction of the two to Mallory's phosphotungstic–acid hematoxylin stain. The pure brown pigment merely retained its brownish color, but the brick red material, even when it was mixed with brown pigment, stained the deep orange color which collagen retains in that stain. This staining reaction of the brick red intracellular material proved of great assistance in searching for the small bits of material in single cells, but it should be emphasized that the reaction was not used or considered as sufficiently specific to permit absolute identification of red material when it lay outside cells in the lumens of the tubules. Therefore, it was not possible to determine how much of the material forming the casts in the cases of longer duration was derived from the excreted hemoglobin and how much from degenerating epithelial cells.

The number and the size of the casts varied widely without relation to the duration of the oliguria. The casts were most numerous in cases 1 and 3 and least numerous in cases 5 and 6. The possibility remained that the amount of cast material depended on the amount of foreign blood the patient had received, but there were not sufficient clinical data in all cases to permit examination of this possible correlation.

*Interstitial Tissue.*—The edema and the leukocytic infiltration of the interstitial tissue were present in the cases with necrosis of the epithelium of the distal convoluted tubules and the collecting ducts. There was no interstitial reaction in the 2 cases that was not associated with evidence of necrosis of the epithelial cells in these two segments. Furthermore, in any case with such epithelial necrosis the edema and leukocytic infiltration took place about the distal convoluted tubules and collecting ducts (fig 3 *B*). There was no degeneration of the lining cells or leukocytic

infiltration of the glomeruli, proximal convoluted tubules or Henle's tubules. When leukocytes appeared in the interstitial tissue beside any of these, a nearby degenerating distal convoluted tubule or collecting duct was found to serve as a satisfactory explanation for the interstitial reaction. Rarely there were occasional leukocytes about a large collecting tubule of the medulla with the lumen filled with cast fragments, leukocytes and the lining epithelium desquamated. It was possible that this truly was a peritubular interstitial inflammatory reaction, but on the basis of the absence of any instance of unmistakable infiltration with large numbers of leukocytes and the absence of any evidence of sequences of epithelial cell necrosis in any tubules of this sort, these findings were judged to be secondary to the presence of the material in the lumen. Therefore, it was concluded that the interstitial reaction was related both anatomically and in time of appearance to the epithelial necrosis in the distal convoluted tubules and the collecting ducts.

There was no evidence that the casts as such were responsible for the degeneration of the tubular epithelium, because this degeneration was present in tubules that contained no casts and, on the other hand, some tubules distended by casts showed no epithelial degeneration. If the casts produced the necrosis of the epithelium by some mechanical effect, this effect should have been produced by the large casts in the collecting tubules of the pyramids. Therefore, the absence of degenerative sequences in these tubules and the absence of inflammatory reaction about them in most cases were strong evidence against the theory that mechanical blockage by casts was the cause of tubular degeneration and interstitial reaction.

*Proximal Convoluted Tubules.*—There was severe cloudy swelling of the epithelium in the proximal convoluted tubules in case 1, but this was not present in the later cases. In all cases these tubules contained circular reticulum, and in all except case 1 they were slightly dilated. It was impossible to attach any great significance to any of these changes. The cloudy swelling disappeared as the duration of the cases increased. It has been shown that circular reticulum can be produced by relatively innocuous measures, such as the feeding of a high protein diet [9] or the feeding of ammonium chloride,[10] without producing renal insufficiency. Finally, slight dilatation of the tubules and reduction in the height of the lining cells have been recognized as normal variations in the histologic picture of active kidneys.[11] On these grounds it seemed permissible to consider that these changes were not the cause or the result of the changes in the other parts of the kidneys.

9. Jackson, H.: Am. J. Path. **3**:285, 1927.

10. Seegal, B. C.: Arch. Int. Med. **39**:550, 1927.

11. Huber, G. C., in Berglund, H., and Medes, G.: The Kidney in Health and Disease, Philadelphia, Lea & Febiger, 1935, p. 12.

*Glomeruli.*—The interpretation given with regard to the proximal convoluted tubules was applied also to the dilatation of the glomerular capsules and to the circular reticulum that appeared in these spaces. Furthermore, there was no evidence of inflammatory or degenerative changes in the vessels of the glomerular tufts.

*Blood Vessels.*—There was slight arteriosclerosis in cases 1, 2, 3 and 6, but on the whole the large and small arteries were well preserved. In cases 2, 4 and 6 one or more large veins contained small mural thrombi. These did not fill the vessels. Each was composed of fibrin and leukocytes alone; in cases 2 and 4 the vessel walls to which the thrombi were attached were not infiltrated with cells. These were interpreted as agonal thrombi. However, in case 5 there were instances of degeneration of a tubule beside a small vein and deposition of fibrin. Both types were interpreted as recent thrombi that did not obstruct the vessels and therefore played no part in the production of the renal changes.

*Comment.*—In the survey of these 7 cases it became evident that the epithelial necrosis and leukocytic infiltration were confined to the distal convoluted tubules and collecting ducts and that the earliest change in the epithelium that was peculiar to these two segments was the occurrence of segregated brick red material in the cell cytoplasm. This was present when no other evidence of degeneration was demonstrable.

This sort of material has been found in the tubular epithelium in a number of different conditions. It was described first by Heineke[12] in a study of the changes in human kidneys after mercury bichloride poisoning. In the cases in which the patients lived for about five days or longer that author found the material surrounded by the cytoplasm of single cells or multinucleated cells. He expressed the belief that these cells were epithelial and that they were phagocytosing (from the tubular lumens) necrotic material that had come from cell degeneration higher up in the tubular system. He expressed the opinion that of all the changes described in mercury bichloride poisoning this epithelial phagocytosis alone was characteristic of that condition. Later, similar intracellular material was described in the kidneys following uranium poisoning.[13] Occasional cells containing "acidophilic hyalin coagula somewhat similar to the picture found in corrosive sublimate poisoning" were reported in the severest lesions of the convoluted tubules in so-called tubular nephritis of children. In this condition, according to Wolbach and Blackfan,[14] the acidophilic material was one of several

12. Heineke, A.: Beitr. z. path. Anat. u. z. allg. Path. **45**:197, 1909.
13. Hunter, W. C.: Ann. Int. Med. **2**:796, 1928.
14. Wolbach, S. B., and Blackfan, K. D.: Am. J. M. Sc. **180**:453, 1930.

morphologic evidences of degeneration of tubular epithelium, but the authors expressed the belief that the important manifestations of so-called tubular nephritis were not consequences of injury specific to the kidneys.

Similarly, in these cases of post-transfusion anuria the resemblance of the necrosis of tubules to that in mercury bichloride poisoning may be discounted, and the important manifestations of the condition may be shown not to be the invariable consequences of the injury seen in the kidney, because entirely similar changes occur without producing renal insufficiency in deeply jaundiced infants.

### COMPARISON WITH KIDNEYS IN DEEP JAUNDICE

All the essential features of the renal changes of the post-transfusion reaction could be found in the kidneys of jaundiced infants with congenital atresia of the bile ducts.[6] In the kidneys of these infants edema and leukocytic infiltration of the interstitial tissue took place only about the distal convoluted tubules and collecting ducts. Necrosis of epithelial cells occurred only in these two segments. The simplest change in these segments was the appearance of bright red material (often tinged with brown) in epithelial cells. Varying numbers of casts, composed of material resembling that in the kidneys of persons showing the reaction to transfusion and often as numerous as those in such kidneys, were present in the distal convoluted tubules and collecting ducts of all the kidneys of the infants and in the collecting tubules in 8 of the 18 cases. In spite of the marked resemblance of the changes in the kidneys of the infants with congenital atresia of the bile ducts to the changes in transfusion uremia, the clinical records of the infants gave no evidence of renal insufficiency. Thus, it seems unlikely that the morphologic changes in the kidneys after delayed reaction to transfusion are responsible for the renal insufficiency.

### SUMMARY

The only progressive renal changes in these cases of post-transfusion uremia are necrosis of the distal convoluted tubules and collecting ducts and infiltration and edema of the interstitial tissue about these segments of the tubules.

This necrosis of epithelium is the determining factor in the final morphologic picture. It is the presence of this necrosis in the distal convoluted tubules and collecting ducts that determines the location of the cellular infiltration of the interstitial tissue. The earliest evidence of this process is the appearance of the brick red material in epithelial cells. This material is regarded as a morphologic manifestation of cell injury. It is possible that the formation of epithelial buds is an even earlier evidence of cellular response to injury, but the absence of such buds in case 2 makes it impossible to confirm this.

The cause of the localized epithelial necrosis remains undetermined. Only when this is known will it be possible to appreciate the full significance of these changes.

There is no evidence that the casts by any strictly mechanical obstruction produce either the oliguria or the epithelial necrosis.

These progressive changes and the other often mentioned features, such as intratubular varicolored casts, are duplicated in the kidneys of jaundiced infants who during life had no oliguria or renal insufficiency. Therefore, it is unlikely that the morphologic changes in the kidneys after delayed reaction to transfusion are responsible for the renal failure observed clinically.

# Case Reports

## ELASTIC TISSUE HYPERPLASIA OF THE ENDOCARDIUM

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PHILADELPHIA

Elastic tissue hyperplasia has been observed in the endocardium of an infant who had not presented clinical evidence of heart disease during the first months of his life. Gross [1] recently reviewed the literature concerning this cardiac anomaly.

### REPORT OF A CASE

A white boy 5½ months of age was admitted Feb. 17, 1941. The mother, 38 years of age, had had one previous pregnancy, four years before, with delivery of an apparently normal girl. Three years preceding her second pregnancy she had right salpingo-oophoritis. She received prenatal care only during the last three months of this second pregnancy; she stated that she had not felt well since a dental extraction three months before this conception. An abscess of the left Bartholin's gland was excised in the sixth month of this gestation period. Her general condition was otherwise not remarkable. The Wassermann test was reported negative. Urethral and cervical smears were reported as morphologically negative for gonococci.

The patient, a full term infant, had been delivered spontaneously and weighed 5 pounds 15 ounces (2,693 Gm.) at birth. The course had been uneventful until the present illness. At no time had there been clinical evidence of heart disease. The mother had noted, however, that the child appeared to be cold in the morning, notwithstanding apparently adequate bed clothing. He had gained satisfactorily on an evaporated milk formula with supplementary foods. Pertussis immunization was carried out in January 1941.

On admission he was acutely ill. The diagnosis was nasopharyngitis, bilateral otitis media and bronchopneumonia. Roentgenograms of the chest showed a large globular heart and a large thymic shadow. There was an enlargement of the cardiac outline to the left with distant heart sounds, without murmurs and without signs of congestive heart failure. Roentgenologically, the heart was markedly enlarged to the left with a loss of waistline and a prominence of the second left arch. The electrocardiogram showed sinus rhythm with a rate of 150 per minute. The P deflections were normal, and the atrioventricular conduction time measured 0.12 second. There was no axis deviation. The T waves were positive in leads I and II. The Q-T interval measured 0.30 second (for children the upper limit of the normal for a cycle length of 0.40 second is 0.27 second).

Bilateral myringotomy was performed. The pus from the ears contained Pneumococcus type 19. Sulfathiazole (2-[paraaminobenzenesulfonamido]-thiazole) was given. Anemia (9.5 Gm. of hemoglobin and 3,920,000 erythrocytes) was partially corrected by transfusions. The blood culture was sterile. The Wassermann test was reported to be negative. Urinalyses gave essentially normal findings.

February 25 he had a probable extension or recurrence of the pneumonia. A roentgenogram showed obstructive emphysema. The carbon dioxide–combining

From the Department of Pathology and the Department of Pediatrics, Temple University School of Medicine.

1. Gross, P.: Arch. Path. **31**:163, 1941.

power was 33 volumes per cent. Alkali and oxygen therapy were instituted; the administration of sulfathiazole was continued.

He died on February 26. The clinical diagnosis at the time of death was bronchopneumonia, suppurative otitis media and a cardiac lesion of undetermined nature. The impression of the roentgenologist was that the cardiac lesion was congenital; that of the cardiologist was that the evidence appeared to be against the possibility of a congenital cardiac lesion.

*Autopsy.*—The body was that of a well developed boy weighing 7 Kg. The skin was extremely white and dry. The pericardial sac was distended with 100 cc. of clear yellow fluid. The heart weighed 75 Gm. and measured 5.5 cm. from

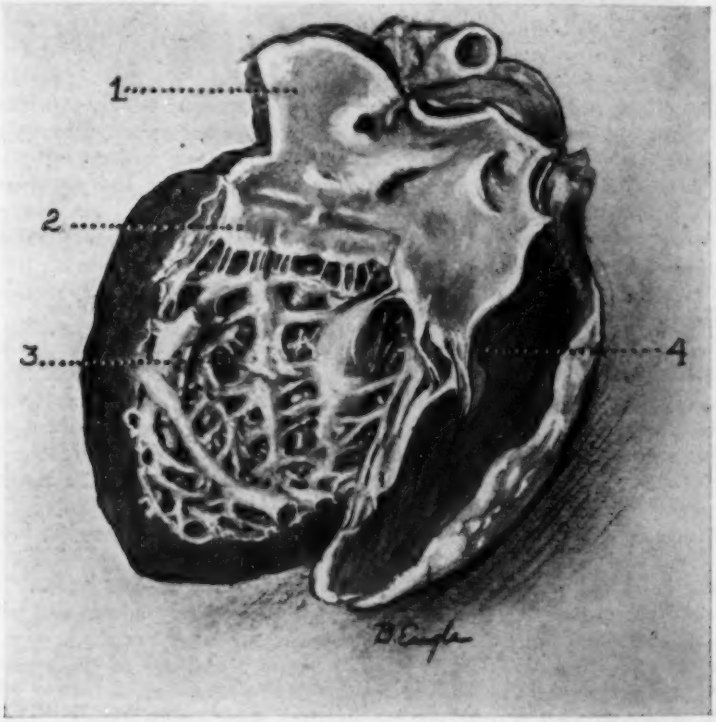


Fig. 1.—Drawing of the left side of the heart: *1*, left atrium; *2*, anterior cusp of the mitral valve; *3*, left ventricular endocardium; *4*, posterior cusp of the mitral valve.

the apex to the base. The insertion of the larger vessels was normal, and none appeared to be hyperplastic or hypoplastic. The ductus arteriosus was not functionally patent. The right atrium was not enlarged; its wall was of normal thickness. The myocardium and the endocardium of the right ventricle showed nothing of significance. The tricuspid and the pulmonic valve were both normal in size and in appearance.

The left atrium and the left ventricle were dilated. The wall of the left atrium measured 0.3 cm. in thickness. The left ventricle measured 3.5 cm. in its widest diameter, and the wall measured 0.7 cm. in thickness. They were both lined with a smooth glistening milky opaque endocardium, which was singularly limited to

the left side of the heart. This thickening appeared grossly to be uniform and did not appear to be scar tissue. The interventricular septum was 0.8 cm. in thickness. The trabeculae carneae were exceedingly prominent. The posterior cusp of the mitral valve was normal, but the anterior cusp was uniformly narrowed to 4 mm., measured from its insertion to its free edge. The chordae

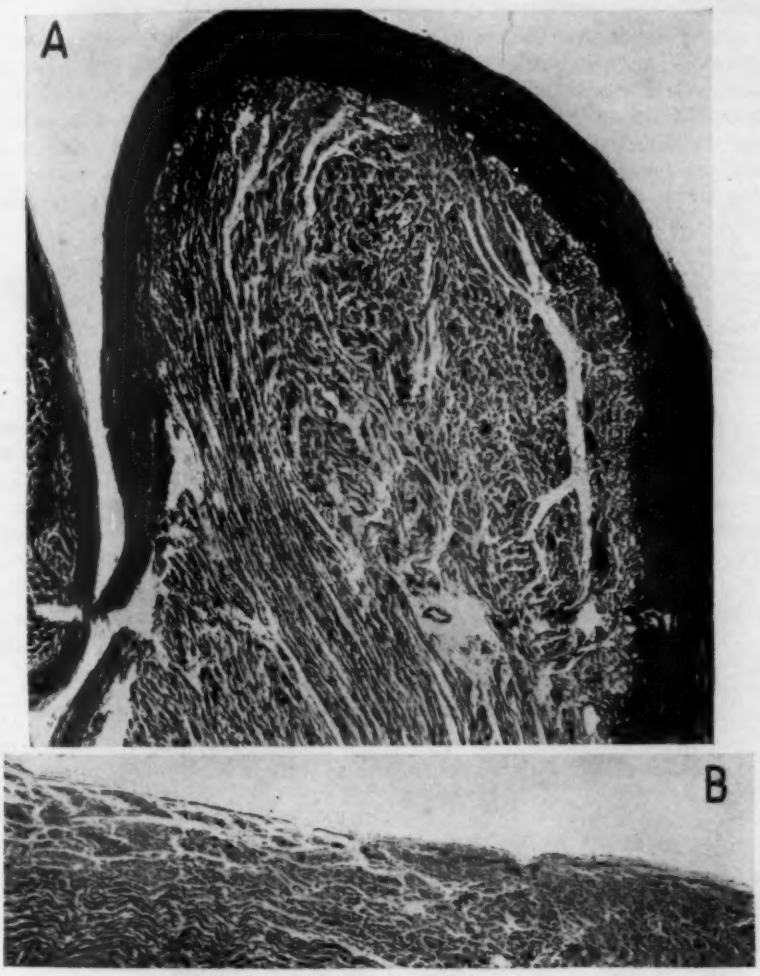


Fig. 2.—*A* shows the left ventricle; Verhoeff's elastic tissue stain. *B* shows absence of hyperplasia of the endocardium in the right ventricle.

tendineae were short, each 0.4 to 0.5 cm. in length, slender, and inserted directly into the ventricular wall. There was no thickening of the cusps of the valve (fig. 1). It was evident from this anatomic disposition that the closure of the mitral valve must have been insufficient.

*Microscopic Observations.*—The histologic examination confirmed the initial impression that the endocardial changes were noninflammatory. By Masson's

stain the lining was uniformly green, and at various points there was extension of the endocardial tissue into the myocardium without retraction. Verhoeff's stain revealed an unusual fibroelastic tissue hyperplasia, similar to that described by Pototschnig[2] (fig. 2*A*). There was no evidence of an inflammatory cellular infiltration. Sections through different parts of the valves showed absence of elastic tissue hyperplasia and of inflammatory reaction. In comparison the endocardium of the right side of the heart showed no change (fig. 2*B*).

There was no free fluid in the pleural cavities. The lungs were congested and edematous; the right weighed 115 Gm. and the left 50 Gm. In both lungs there were several areas of healing bronchopneumonia. The spleen was enlarged, and there was follicular hyperplasia. The mesenteric lymph nodes were hyperplastic, and the follicles of the gastrointestinal tract were accentuated; they showed moderate lymphoid hyperplasia and reticular hyperplasia of unusual degree, probably inflammatory in nature. An unusually large parathyroid gland was normal histologically.

Four months later, the father of this patient died at another hospital. The pathologic diagnosis was acute ulcerative endocarditis with left ventricular hypertrophy, mesenteric and retroperitoneal lymphadenopathy and splenic infarct. The Wassermann test was reported to be positive. Unfortunately, tissue was not available for special staining in order that it might be compared with specimens of the infant's heart, and blood had not been obtained for culture.

The cause of the endocardial hyperplasia remains obscure. In some of the cases reported previously, different infectious processes were noted, some of which occurred synchronously; in many other instances no infection was noted. Syphilis, though it has been present rarely, has been included as a possible etiologic factor. In this case the Wassermann test was reported negative for both the mother and the child; however, the father's blood gave a positive reaction. It is our impression that the process was developmental rather than infectious.

In two other autopsies, by other members of the pathologic staff, mild hyperplasia of the elastic tissue of the endocardium of the left side of the heart was noted. In one of the subjects the condition had been previously suspected of being tuberculous, but this was not confirmed histologically. Subsequent reexamination with special stains revealed the same elastic tissue hyperplasia of the endocardium as well as an identical mitral pathologic anatomy. The ages of the children were 5½ years and 2 years, respectively.

Elastic tissue stains were performed on the cardiac tissue in a case of rheumatic endocarditis and on that in a case of endocardial fibrosis secondary to infarction, but the type of elastic tissue hyperplasia described here was not noted.

## SUMMARY

A case of elastic tissue hyperplasia of the endocardium of the left side of the heart, associated with narrowing of the anterior cusp of the mitral valve, in a 5½ month old boy who had not shown evidence of cardiac disease before his terminal illness is reported. Similar findings have been noted in 2 other patients.

Elastic tissue hyperplasia was not found in the cardiac tissue in a case of rheumatic endocarditis or in a case of endocardial fibrosis secondary to infarction.

Temple University Hospital.

2. Pototschnig, G.: Ztschr. f. ang. Anat. **4**:234, 1919.

# PRIMARY CARCINOMA IN A CONGENITALLY SINGLE FUNCTIONING KIDNEY

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Congenital absence of one kidney is not rare, and numerous abnormalities of the remaining kidney have been reported. Thompson [1] regards the functioning kidney in such cases as peculiarly liable to infection. No report of primary carcinoma in such a kidney demonstrated at operation or at autopsy could be found in the literature.

## REPORT OF A CASE

The patient when first admitted was a well developed, well nourished 49 year old white man. He complained of gross hematuria of two weeks' duration, intermittent at first, later constant; a slight loss of weight, and a "tired feeling" for several months. His past history and the family history were noncontributive. A roentgen diagnosis of tumor was made. Hypernephroma of the right kidney was suspected, with nonfunction of the left kidney. He returned six months later because massive hemorrhage from the kidney had caused the formation of blood clots in the bladder and blocked the urethra. Until his death, a year and a half later, blood cells were found in the urine on every microscopic examination. Occasionally there was gross hemorrhage. This usually followed some unusual strain. The patient was able to continue with his work most of the time for a year following his first severe hemorrhage. Then he suffered a second severe hemorrhage. He was admitted to the hospital two days later. His right kidney could now be palpated for the first time. After the hemorrhage was controlled, he left the hospital. An inability to retain food gradually developed, and the patient became progressively more anemic and cachectic. He died at home four months later.

*Autopsy.*—Except for extreme emaciation, nothing of unusual note was found outside of the genitourinary tract. The right kidney was large, with irregular bulging shallow nodules over the midportion. Its capsule was moderately thickened and fibrosed, but the organ was easily separated from the surrounding tissues. It measured 11 by 12.5 by 19 cm. and weighed 1,300 Gm. In the midportion there was a large, well circumscribed tumor, roughly ovoid, measuring 9 by 12.5 by 13 cm. (fig. 1). Grossly it appeared to be encapsulated except for a portion protruding into the pelvis, a portion ragged and friable and covered with clotted blood. The tumor was composed of small islands of soft yellow tissue enclosed by wide, firm, translucent gray bands, and numerous areas of cystic degeneration. The largest of these cysts was 3 by 4.5 by 6 cm. There were numerous small hemorrhages. There was no gross invasion of the larger renal veins by the tumor. At each pole of the kidney was a portion of relatively normal renal tissue. The cortex of these portions measured between 1.5 and 2 cm. in thickness, and the medulla between 2 and 2.5 cm., indicating that the kidney had become extremely hypertrophied. The pelvis measured 9 cm. in length. The kidney extended well up beneath the liver, which had become compressed and thinned over it. The right

From the laboratory of the Ball Memorial Hospital, Muncie, Ind.

1. Thompson, A. R.: Guy's Hosp. Rep. **79**:297, 1929.

ureter was not dilated. Both adrenals were normal in size. A large, dilated ureter, irregular in diameter and somewhat tortuous, was found on the left side. By tracing the blood supply of the left adrenal, rudimentary renal vessels were found which led to a small nodule, consisting chiefly of fat and fibrous tissue,

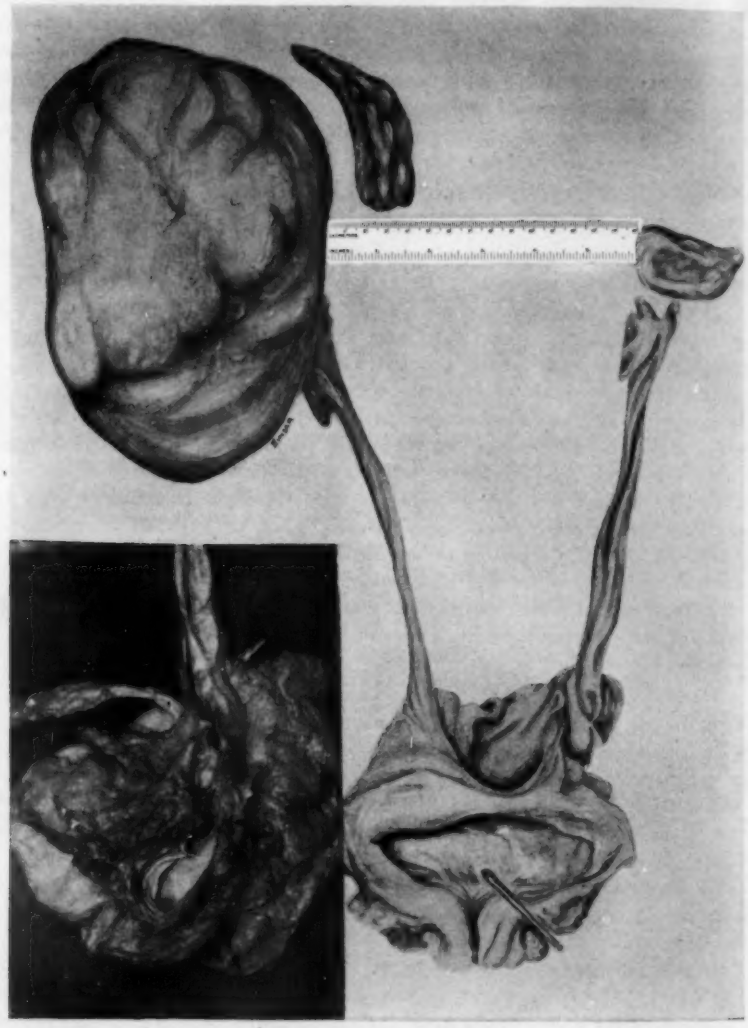

Fig. 1.—Gross specimen. In the bladder a probe is in the single ureteral opening. At the top of the left ureter is the small fibrous nodule containing tubules. Above it is the normal size adrenal. Inset: Posterior surface of the urinary bladder showing the saccular ending of the left ureter.

which measured 0.5 by 1.5 by 2 cm. It was attached to the top of the left ureter by a narrow cord in which a lumen could not be found. The ureter was 24 cm.

long. It ended as a blind pouch on the posterior surface of the bladder. The ureter and pouch were filled with a light brown thin clear fluid. Several small cysts from 3 to 5 mm. in diameter in the lining epithelium of the ureter were filled with the same fluid. No chemical analysis of the fluid was attempted because the body had been embalmed, but the presence of cysts in the wall of the ureter filled with the same fluid indicated that the fluid was not necessarily drainage from the nodule. There was no trigon in the bladder. The right ureteral orifice was in the midline. (In figure 1 a probe is in the right ureteral orifice.) No left ureteral orifice was found or any indication that one had ever been present. The right seminal vesicle was present and appeared to be normal, as was the vas deferens. Medial to the distal end of the left ureter there was an ovoid cystic mass, 1.5 by 3 cm. in diameter, which on microscopic section proved to be a dilated seminal vesicle. It was filled with a thick brown fluid. The left vas deferens was not present. There was a tubelike portion of the cyst which was suggestive of a vas deferens, but it ended blindly. No connection was found between the cyst and the ejaculatory duct.

Metastases were not found in any internal organs.

Microscopically, the tumor was composed chiefly of large masses of clear cells, roughly cuboid, arranged in cords and strands, and surrounding small sinus-like spaces, many of which were filled with blood. In large areas the cells were fairly uniform in size. There was, however, considerable variation in the size, shape and chromatin content of the nuclei. In other places the cells were more elongated, but still surrounded sinus-like spaces. In a few places the cells were almost spindle shaped and grew in irregular masses. The latter cells showed a more deeply staining cytoplasm. Very few mitotic figures were found. Nucleoli were inconspicuous. In many sections well defined fibrous bands separated nodules of tumor tissue. While grossly in many places there appeared to be a capsule between the tumor tissue and the renal parenchyma, on microscopic section this zone was found to be composed of compressed tubules and glomeruli, and in some regions small islands of tumor cells were found in the renal parenchyma. In sections from portions of the tumor which appeared grossly to be solid there were areas of necrosis and hemorrhage. A diagnosis of adenocarcinoma of the kidney was made.

The nodule of tissue at the head of the left ureter contained a few dilated spaces lined with a pseudostratified columnar epithelium and a few clustered spaces lined with low cuboidal epithelium, which resembled collecting tubules. Most of these appeared to be empty. A few of them were filled with a structureless homogeneous material staining with eosin, possibly hyalinized debris. The entire fragment was embedded in paraffin and numerous sections made, but nothing resembling a glomerulus or an excretory tubule could be found. Evidently the entire nodule was a part of the ureteral bud and never had joined the nephrotome.

Gutierrez [2] compiled the results of 92,650 autopsies reported by various authors and gave the relative frequency of hypoplastic kidney as 1:60, that of renal aplasia as 1:400 and that of congenital solitary kidney as 1:600. In a survey of the records of 2,276 autopsies at the Children's Memorial Hospital, Chicago, over a period of nineteen years, I found 72 cases of congenital anomalies of the urinary tract. There were 4 cases of congenital absence of one kidney, 5 cases of renal aplasia and 3 cases of renal hypoplasia. The ages varied from 10 days to 10

2. Gutierrez, R.: Arch. Surg. **27**:686, 1933.

years; most of them, however, were 1 year or less. While the incidence of congenital absence of one kidney and that of aplastic kidney are closely approximate to the figures for all age groups compiled by Guiterrez, the incidence of hypoplasia is far lower, 1:758.

Most reports of renal carcinoma are surgical reports, and fail to give relative frequencies. However, Bell [3] reported 149 cases of carcinoma of the parenchyma of the kidney and 3 cases of carcinoma of the pelvis in 30,000 autopsies; McWhorter and Cloud [4] reported 42 cases in 13,500 autopsies, this number including 32 cases in which the neoplasm was diagnosed as hypernephroma. According to these figures, 194 cases of carcinoma of the kidney were found in 43,500 postmortem examinations, or an incidence of slightly less than 1 in 225 autopsies. One might reasonably expect to find carcinoma in an aplastic or a solitary kidney in from 100,000 to 135,000 of persons examined post mortem. How-

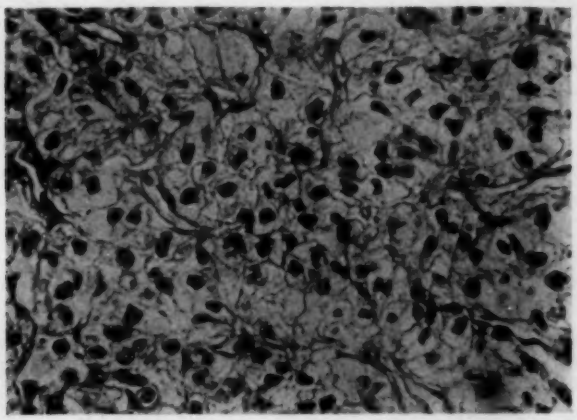

Fig. 2.—Section representative of the major portion of the tumor tissue.

ever, no report could be found of such a finding verified at operation or autopsy. Mintz and Stewart [5] reported a case of solitary kidney invaded by carcinoma primary in the stomach. Burrell [6] reported a case which he diagnosed as one of carcinoma in a solitary kidney by roentgen examination. He likewise was unable to find a report of a case of cancer in a congenitally single functioning kidney.

SUMMARY

A case of primary carcinoma in a congenitally single functioning kidney is reported. Extensive search has been made of the literature covering both renal anomalies and renal carcinoma. No report of a similar case, with the diagnosis confirmed by surgical exploration or by autopsy, was found.

Children's Memorial Hospital.

3. Bell, E. T.: J. Urol. **39**:238, 1938.
4. McWhorter, J. E., and Cloud, A. W.: Ann. Surg. **92**:434, 1930.
5. Mintz, E. R., and Stewart, J. D.: New England J. Med. **205**:1282, 1931.
6. Burrell, N. L.: Urol. & Cutan. Rev. **40**:312, 1936.

# Laboratory Methods and Technical Notes

## ROUTINE STAINING OF THE BETA CELLS OF THE ISLETS OF LANGERHANS WITH MASSON'S TETRACHROME STAIN

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Since the fundamental studies of Lane and Bensley were published,[1] several methods for the differential staining of the cells of the islets of Langerhans of the human pancreas have been devised. Those of Bloom [2] and Goodpasture [3] have the disadvantage that fixation in Zenker's solution with or without neutral formaldehyde solution is necessary, and this fixation cannot always be used in routine postmortem work. The methods of Gomori [4] give excellent results, but require very thin sections and prolonged mordanting and thus are more suited for special research work than for the daily routine work of the pathologist; who needs a method which is applicable to material fixed in routine fixatives and to sections of normal thickness and which does not require too much time.

By chance, in the laboratory of the Public Health Service at Curaçao it has been found that the Masson tetrachrome stain in the modification of Larson and Levin [5] is a good stain for the beta cells of the islets and differentiates them clearly from the other cell types. As we obtained constant results with this method and the stain is excellently suited for the simultaneous staining of large numbers of sections, we felt that our routine might be useful to others.

To obtain good results, however, some precautions have to be taken. The material must be well fixed and embedded with some care. In our laboratory the following methods are used.

### METHODS

*Fixation.*—In the first place a mixture of equal parts of Bouin's fluid and a saturated aqueous solution of mercury bichloride is used. The duration of fixation in this fluid is twelve to twenty-four hours. Bouin's fluid and Heidenhain's mercury bichloride–formaldehyde mixture also give excellent results. The slices of pancreatic tissue, cut for fixation, should not be thicker than 3 mm.

*Embedding.*—Péterfi's methylbenzoate-celloidin method for paraffin embedding is routinely used and is in our opinion much superior to the different dioxane technics, which cause much more shrinking.

1. Romeis, B.: Taschenbuch der mikroskopischen Technik, Munich, R. Oldenbourg, 1932, p. 627.
2. Romeis,[1] p. 628.
3. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938, pp. 209-212.
4. Gomori, G.: Am. J. Path. **15**:497, 1939; **17**:395, 1941.
5. Larson, C. P., and Levin, E. J.: Arch. Path. **29**:272, 1940.

*Thickness of Sections.*—Sections of 6 microns are thin enough and can always be obtained in our climate.

After removal of the trinitrophenol and the mercury precipitate, the sections are stained as prescribed by Larson and Levin, the only difference being that we substitute light green by fast green F. C. F.

The result is that the beta cells are seen to be filled with closely packed red-staining granules and contrast sharply with the other islet cells, which show a pale brownish color. The difference in staining reaction can easily be observed with low magnification.

# Forensic Medicine

## CHANGES IN OXYGEN SATURATION OF ARTERIAL BLOOD INCIDENT TO DEATH FROM SUDDEN CARDIAC FAILURE OR ASPHYXIA

### AN EXPERIMENTAL INVESTIGATION

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The phenomenon of sudden death is invariably a matter of medical and frequently a matter of medicolegal interest. In most instances sudden death is the result of rapid and unexpected failure either of circulation or of respiration. Although the cause of such a dramatic functional breakdown is often disclosed by postmortem examination, this is not invariably the case. In many instances a cardiac or a cerebral lesion which would not in other circumstances be regarded as incompatible with life is designated as the probable cause of death. In such circumstances the evidence bearing on the actual mechanism of death is likely to be inconclusive. Failure to find any significant organic change at autopsy sometimes leads to the making of such unsatisfactory pathologic diagnoses as "status thymicolymphaticus" or "primary shock."

This investigation was undertaken for the purpose of learning whether or not in certain cases the degree of oxygen saturation of a postmortem sample of arterial blood might not provide some evidence as to the nature of the terminal functional failure. It seems reasonable to suppose that if death occurred as a result of unexpected asystole or ventricular fibrillation, the oxygen saturation of the arterial blood at the moment of death might be relatively high. On the other hand, if the heart stopped because of systemic asphyxia, it seems likely that the oxygen saturation of the arterial blood at the moment of death would be found low. From a medicolegal standpoint, knowledge of the degree of oxygen saturation of arterial blood at the moment of death might conceivably provide valuable evidence of a confirmatory or of an exclusionary nature. Since in medicolegal autopsies the interval between death and postmortem examination is often variable, information concerning the rate and the character of postmortem changes in the oxygen concentration of arterial blood would obviously be desirable.

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## EXPERIMENTAL PROCEDURE

Adult dogs were used throughout the experiments. The animals were killed in various ways, including the production of mechanical asphyxia by obstruction of the trachea, the production of chemical asphyxia through narcosis induced with soluble pentobarbital U. S. P. and the production of instantaneous cardiac arrest by direct electric shock. Blood was taken before and at various intervals after death, and its oxygen saturation was determined.

Sterile needles, cannulas and instruments were used, and all injections and punctures were made through skin previously shaved and wiped with iodine and alcohol. Precautions were taken to insure that no air would enter the heart or the blood vessels either during the sampling or in the intervals between sampling.

The blood samples were taken from the animal into a syringe containing liquid petrolatum and transferred immediately without contact of air to oxalate tubes containing a ¾ inch (2 cm.) layer of liquid petrolatum, and the tubes were then transferred to a refrigerator to await analysis, which was done as soon as possible after the sample had been taken.

*Technics Used in Obtaining the Blood Samples.*—When a series of samples were to be taken from the same chamber of the heart, a cannula with a tight fitting stylet was introduced through the chest wall into the desired cardiac ventricle, the sample taken, the stylet replaced and the projecting end of the cannula then sealed with melted paraffin or sealing wax. In all such cases the true position of the cannula was later checked by postmortem examination. Often the left common carotid artery was cannulated with similar precautions. When only one sample was required from a chamber of the heart, this could be obtained through a fine needle, which was then immediately withdrawn. At postmortem examination the puncture hole was identified to confirm the correct origin of the blood sample. The final cardiac samples might then be taken under direct vision after the chest had been opened, the trachea having previously been clamped to prevent collapse of the lungs, which might force into the heart blood that had actually been in the lungs. In the later samples sedimentation of the red blood cells often made adequate sampling difficult. It was with such samples that calculation of the percentage of oxygen saturation was of particular value.

In experiments of long duration and in all the cases of nonasphyxial death, in which the clotting tendency was much greater, a dose of 2 to 3 cc. of liquaemin (a heparin preparation) was given intravenously immediately before the animal was killed.

*Analysis of Blood Samples for Oxygen Content and Oxygen Capacity.*—The usual manometric Van Slyke-Neill technic was used. Briefly, this consisted in extracting the gases from a 1 cc. sample of the blood introduced into the Van Slyke machine without any exposure to the air, separately absorbing the carbon dioxide and oxygen evolved, and, from the manometrically measured gaseous pressure differences, calculating the oxygen content of the sample in terms of cubic centimeters of oxygen per hundred cubic centimeters of blood (volume per cent). The oxygen capacity was determined by equilibrating the blood with atmospheric air in a small tonometer for five minutes or more while taking considerable precautions to prevent fluid loss, and then determining the amount of oxygen contained in this fully arterialized blood. The oxygen content of the original sample divided by its oxygen capacity and expressed as a percentage is of course the percental oxygen saturation of the blood sample.

### THE OXYGEN CONTENT OF THE BLOOD AFTER MECHANICAL ASPHYXIA

Two dogs were used in these experiments. Light anesthesia was induced by the intraperitoneal injection of soluble pentobarbital U. S. P. in saline solution. Acute mechanical asphyxial death was then produced by clamping off the trachea. Blood samples were taken from both animals immediately after their hearts stopped beating. One animal was allowed to remain at room temperature (65 F.) for a period of two and three-quarter hours, at which time further blood samples were taken. The other animal was placed in a refrigerator at a temperature close to freezing (33 F.), and subsequent samples were taken five and three-quarter hours after death.

TABLE 1.—*The Oxygen of the Blood After Mechanical Asphyxia*

| Time After Death | Source of Sample | Oxygen Content, Vols. % | Oxygen Capacity, Vols. % | Percental Saturation, Uncorrected |
|---|---|---|---|---|
| Dog 1. Mechanical Asphyxial Death—Allowed to Remain at Room Temperature | | | | |
| Immediately | Left common carotid artery..... | 0.73 | ? 23.28* | 3.14 |
| | Left ventricle..... | 0.19 | ? 23.28 | 0.82 |
| | Right ventricle..... | 0.34 | 23.28 | 1.46 |
| 2¾ hours | Left ventricle..... | 1.11 | ? 23.28 | 4.77 |
| | Right ventricle..... | 0.00 | ? 23.28 | 2.56 |
| Dog 2. Mechanical Asphyxial Death—Placed in Refrigerator at 1.0 C. | | | | |
| Immediately | Left ventricle..... | 0.78 | 25.40 | 3.07 |
| 5¾ hours | Left ventricle..... | 0.22 | 25.65 | 0.86 |
| | Right ventricle..... | 0.12 | 10.78 | 1.13 |

* The question mark indicates an assumed capacity, not actually measured.

The sources of these various samples and their oxygen contents, capacities and saturations are given in table 1. It may be mentioned here that small variations observed in the oxygen contents of the blood samples are not significant in that they may be due in part to slight variations in the hemoglobin content of the different samples, resulting from natural sedimentation of the red cells, and in part to the relatively more apparent and larger analytic error consequent to such low oxygen contents. The term "Uncorrected" in the last column of the table means that the percental saturation was calculated from the total oxygen content and total oxygen capacity of the sample, no attempt being made to differentiate the oxygen combined with hemoglobin from that in simple physical solution in the plasma.

It will be noted from this table that in the animals dying of acute mechanical asphyxia the oxygen content of the arterial and venous blood was reduced to more or less identical and extremely low values. As in both dogs death occurred within three minutes after the clamping of the

trachea, the marked ability of the tissues to extract oxygen from the blood even at very low partial pressures of oxygen is strikingly illustrated. These low values indicate that a high pressure head of oxygen in the blood is not necessary in order that oxygen may pass readily from the blood to the tissues.

The rationale of the experiment in which the dog was refrigerated was to determine whether or not when the usual postmortem putrefactive processes were inhibited by the low temperature, the blood might again gain oxygen by some process of diffusion from without. In order to facilitate such diffusion into the lungs the clamp which had been placed on the trachea was removed during the period of stay in the refrigerator. It is seen that no such gain in oxygen occurred, at least in the blood contained in the ventricles of the heart; instead, still further diminution in the oxygen content is indicated. It would seem, then, that further investigation might prove a cardiac blood oxygen content of significantly

TABLE 2.—*The Action of the Blood After Chemical Asphyxia*

| Time After Death | Source of Sample | Oxygen Content, Vols. % | Oxygen Capacity, Vols. % | Percental Saturation, Uncorrected |
|---|---|---|---|---|
| Immediately | Left common carotid artery | 0.94 | 20.72 | 4.53 |
| | Right ventricle | 0.46 | 20.90 | 2.20 |
| 4½ hours | Right ventricle | 0.53 | 26.42 | 2.00 |

more than 1 volume per cent to be incompatible with death by uncomplicated mechanical asphyxia.

### THE OXYGEN CONTENT OF THE BLOOD AFTER CHEMICAL ASPHYXIA

A 24 Kg. dog was given 2.0 Gm. of soluble pentobarbital intraperitoneally, which resulted in marked respiratory depression, cyanosis and death in a matter of fifteen to twenty minutes. Respiration ceased before the heart beat stopped. Blood samples were taken immediately after death from the left common carotid artery and the right ventricle, and again, after the animal had lain for four and one-half hours at room temperature, from the left and right ventricles.

The results of this experiment are given in table 2. It is seen that the oxygen content of the venous and arterial blood became reduced at death to levels of less than 1.0 volume per cent, levels strictly comparable with those produced by the more acute mechanical asphyxia. This reduced blood did not again gain oxygen during four and one-half hours after death. It thus appears to be the case that in progressive anoxic

anoxia with depression of the respiratory center to the point of extinction and of much slower development than acute mechanical asphyxia, the tissues continue to abstract oxygen almost to the point of its disappearance from the blood. The suggestion would seem to be that when the cardiovascular system is normal, death from respiratory failure is characterized by more or less identical arterial and venous blood oxygen contents of less than 1.0 volume per cent.

### THE OXYGEN OF THE BLOOD AFTER ELECTRICALLY INDUCED CARDIAC DEATH

Acute cardiac death was induced in the following manner. The animals were lightly anesthetized with soluble pentobarbital U. S. P. in small doses injected intraperitoneally, and a needle electrode was inserted through the skin of the right axilla and through the left side of the chest to make contact with the surface of the heart. An electric shock was then given with a 110 volt alternating current, which arrested cardiac function and caused death after a few gasping respirations.

Seven dogs were used in these experiments, which extended over a period of months from winter into summer. No attempt was made to control the temperature of any animal; the dogs were simply allowed to remain at room temperature after death. It soon became apparent that the rate of disappearance of oxygen from the blood after death was influenced considerably by room temperature. The approximate room temperatures are therefore included in the list of observations.

In table 3 are given the results obtained with the blood samples from the 7 experimental animals of this group. It will be noted that in 6 of the cases the oxygen saturation of the blood samples obtained from the left and right sides of the heart immediately after death showed essentially the values and differences which would be found in the normal animal during life, namely, about 90 per cent saturation on the left side and about 60 per cent on the right. The saturation values of dog 10 were much lower, owing to the fact that immediate cardiac death was not produced; two separate shocks were required to produce permanent cardiac arrest, while respiration was inhibited during the intervening moments by the effects of the first shock.

On examining the rate of disappearance of oxygen from the cardiac blood of these animals as estimated from the fall in the oxygen content and percental oxygen saturation of the blood samples obtained at varying intervals up to nine and one-half hours after death, it will be noted (from table 3) that while no 2 animals showed exactly the same rate of oxygen disappearance, the general trend was definite enough; furthermore, several factors influencing the rate of oxygen loss were quite clearly indicated in the course of these experiments. It is seen that when the dead

animal was allowed to remain in an environmental temperature of about 65 F. the oxygen saturation values of the cardiac blood became lowered by about 50 per cent in a period of from six to nine hours. The rates of oxygen loss from the right and left sides of the heart were similar, although the losses on the right side began at a lower level of oxygen saturation. When the environmental temperature was higher, the rate of disappearance of oxygen was much more rapid, and in the case of dog 9, at an environmental temperature of around 82 F. the percental

TABLE 3.—*The Oxygen of the Blood After Electrically Induced Cardiac Shock*

| Dog | Time After Death | Source of Sample | Oxygen Content, Vols. % | Oxygen Capacity, Vols. % | Percental Saturation, Uncorrected | Room Temperature, F. |
|---|---|---|---|---|---|---|
| 3 | 10 min. | Right ventricle | 10.48 | 22.17 | 47.3 | ±65 |
| | | Left common carotid artery | 18.78 | | | |
| 4 | 10 min. | Left common carotid artery | 21.81 | 23.94 | 91.1 | ±65 |
| | 20 min. | Left ventricle | 19.34 | 20.70 | 93.4 | |
| | 15 min. | Right auricle | 16.11 | 25.41 | 63.4 | |
| | 5½ hr. | Left common carotid artery | 8.67 | 21.02 | 41.2 | |
| | 5½ hr. | Left ventricle | 13.68 | 21.52 | 63.5 | |
| | 5½ hr. | Right auricle | 7.42 | 30.22 | 24.5 | |
| 5 | 10 min. | Left common carotid artery | 19.71 | 22.83 | 86.3 | ±65 |
| | 20 min. | Left ventricle | 17.13 | 19.45 | 88.1 | |
| | 15 min. | Right auricle | 13.15 | 23.00 | 57.2 | |
| | 8½ hr. | Left ventricle | 1.85 | 4.62 | 40.1 | |
| | 8½ hr. | Right auricle | 6.58 | 25.82 | 25.5 | |
| 7 | 5 min. | Left common carotid artery | 17.30 | 21.38 | 80.9 | ±65 |
| | 9½ hr. | Left ventricle | 3.92 | 14.39 | 27.2 | |
| 8 | 5 min. | Left common carotid artery | 17.51 | 19.55 | 89.6 | ±76 |
| | 3¾ hr. | Left femoral artery | 4.28 | 15.92 | 26.9 | |
| | 6 hr. | Left auricle | 4.90 | 23.20 | 21.1 | |
| | 6 hr. | Abdominal aorta | 0.21 | 15.25 | 1.4 | |
| 9 | 10 min. | Left common carotid artery | 18.51 | 20.62 | 89.7 | ±82 |
| | 1¼ hr. | Left common carotid artery | 22.15 | 26.71 | 82.9 | |
| | 2¼ hr. | Left common carotid artery | 12.86 | 27.94 | 46.0 | |
| | 3¾ hr. | Left auricle | 4.89 | 28.89 | 16.9 | |
| 10* | 5 min. | Left common carotid artery | 8.29 | 19.36 | 42.8 | ±67 |
| | 5 hr. | Left ventricle | 2.35 | 32.42 | 7.2 | |
| | 5 hr. | Left auricle | 0.65 | 33.41 | 1.9 | |
| | 5 hr. | Right auricle | 0.48 | 20.94 | 2.3 | |

* Two shocks were required to produce death in this animal; hence death was not immediate.

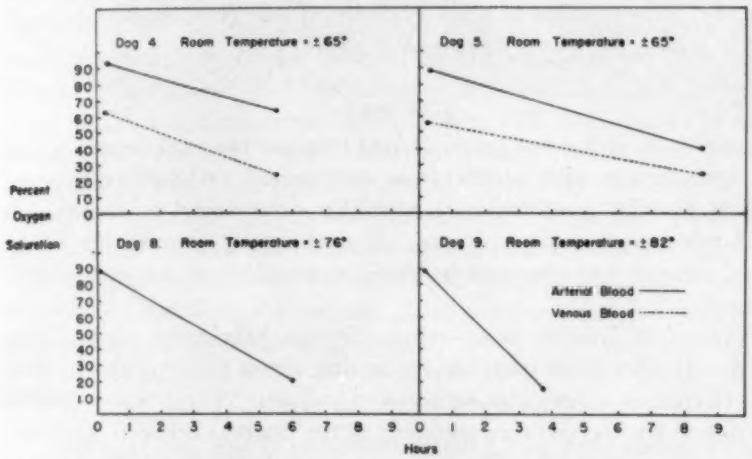
oxygen saturation fell from about 90 per cent to about 17 per cent in three and three-quarter hours.

The effect of temperature on the rate of disappearance of oxygen from the blood and the parallelism between the oxygen losses from the right and left sides of the heart are graphically represented in the accompanying chart.

That blood has a metabolism of its own was demonstrated by Warburg,[1] Morawitz[2] and others by measuring the oxygen consumption

1. Warburg, O.: Ztschr. f. physiol. Chem. **76**:331, 1912.
2. Morawitz, P.: Arch. f. exper. Path. u. Pharmakol. **60**:298, 1909.

of incubated blood preparations. In order to estimate in these experiments how much of the observed changes in oxygen content of the blood samples was due to changes occurring in the blood itself, several different types of measurements were made. In the case of 2 animals of this group (dog 5 and another not listed) blood samples were taken just before death from the left common carotid artery and placed in a stoppered test tube under about 1½ inches (3.8 cm.) of paraffin oil, and the test tube was placed in the abdominal cavity of the animal immediately after death so that it would be exposed to the same temperature conditions as the blood in the heart and blood vessels of the cooling animal. On removal for analysis eight and one-half and nine hours after death, respectively, their oxygen saturation was found to be 83 per cent and 84 per cent, indicating


Dog 4. Room Temperature = ± 65°
Dog 5. Room Temperature = ± 65°
Dog 8. Room Temperature = ± 76°
Dog 9. Room Temperature = ± 82°
Percent Oxygen Saturation
Arterial Blood
Venous Blood
Hours

The postmortem fall in percental oxygen saturation of arterial and venous blood at various environmental temperatures.

a probable oxygen loss of about 12 per cent. That these observations were not complicated by diffusion of oxygen into or out of the tubes was made relatively certain by other experiments in which similarly protected samples of blood already several hours old of 2, 3, 7 and 43 per cent oxygen saturation were left at room temperature and found to show no appreciable change in oxygen saturation over a period of eight hours.

The importance of the source of the sample was clearly indicated in the case of dog 8, in which samples taken from the femoral artery and from the abdominal aorta showed a much more rapid fall in oxygen saturation after death than did the cardiac samples from this and other animals. These findings together with the findings on stoppered blood samples described in the foregoing paragraph strongly suggest that only

a minor part of the oxygen loss from the blood is due to oxidative changes in the blood itself, the major loss being due to diffusion of oxygen outward from the vessels to be utilized in the oxidative processes in the adjacent tissues. The rate of oxygen loss was much less in the thick-walled cardiac ventricles than in the femoral artery and the abdominal aorta. The fact that blood in the relatively thin-walled auricles approximated closely that of the ventricles in rate of oxygen loss suggests that the close proximity of the gas-filled putrefying intestine may have been an important cause of oxygen loss from the abdominal aorta and the femoral artery. The rate of oxygen loss from the smaller vessels of the extremities was not assessed.

In all these experiments blood smears were made from representative samples obtained at various intervals after death and examined microscopically. These failed to show evidence of any gross multiplication of bacteria within the blood stream after death.

## SUMMARY

Samples of cardiac and arterial blood removed from previously normal dogs immediately after death from mechanical (tracheal clamp) or chemical (soluble pentobarbital) asphyxia were found to contain less than 1 volume per cent of oxygen. No subsequent postmortem rise in oxygen content was observed in blood removed from the ventricles of the heart.

Samples of arterial blood removed from previously normal dogs immediately after death from sudden cardiac arrest (electric shock) were found to contain a high concentration of oxygen. There was a gradual reduction in the oxygen concentration as the interval between death and sampling was increased. The rate of oxygen loss was greater in dogs kept at high temperatures than in those kept at low temperatures after death. Oxygen is best retained by blood within the chambers of the heart.

These experiments indicate that postmortem evidence pertaining to the mechanism of death may be secured in certain cases by determining the oxygen saturation of cardiac blood.

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# General Reviews

## ALVEOLAR CELL TUMOR OF THE HUMAN LUNG

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The microscopic picture and the histogenesis of the primary bronchogenic tumors of the lung are comparatively well established, but this is not true of the tumors considered to arise in the pulmonary alveoli. Some authors have firmly believed in the existence of such tumors (Ewing; Oberndorfer [a]; Weissmann and others); some, in spite of broad experience, have never observed them (Pässler; Kraft; D'Aunoy, Pearson and Halpert); and others have flatly denied their existence (Eismayer; Fried [a]; Geschickter and Denison; Arkin and Wagner; Frissell and Knox). This review, consequently, deals with a subject the very existence of which is debatable, and in this respect differs from the usual general review encountered in this journal. We shall endeavor to describe impartially the present state of knowledge and opinion concerning the type of tumor in question.

### DEFINITION

Tumors of this type have been designated by a great variety of terms, among them, "primary multiple carcinoma," "multiple nodular carcinoma," "diffuse lung carcinoma," "alveolar carcinoma," "alveolar cell carcinoma," "carcinosis," "carcinomatoides alveogenica multicentrica" and "alveolar cell tumor." The latter term appears preferable because it leaves open the question of the histogenesis of the tumor cells. We shall discuss this later. The term should not be confused, however, with such other designations as "alveolar carcinoma" or "alveolar sarcoma," which refer to certain tumor structures having no relation to the pulmonary alveoli. Tumors of the type which we are about to consider should exhibit the following major characteristics to distinguish them from the bronchogenic lung tumors: independence of the bronchial system and evidence of multiple or diffuse intra-alveolar origin and growth. Needless to say, primary cancer elsewhere in the body must also be excluded.

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MORPHOLOGY

*Historical Note.*—Malassez in 1876 was the first to describe the multiple nodular form of the alveolar cell tumor. A 47 year old woman fell ill one month before death with dyspnea as the main symptom. A pleuritic exudate developed, and the patient died in asphyxia. At autopsy the left pleural cavity contained fluid, and the right was obliterated. Both lungs were studded with pale red or yellow nodules of pea to cherry size, many coalescent. All other organs were free from tumor involvement grossly, as were the bronchi as far as could be determined. Microscopic examination of the tumor nodules revealed the stroma made up of the alveolar walls, the neoplastic lining cells being cylindric, cuboid and in some cases flat. Papillary protrusions of these cells were common, and in some fields the cells were exfoliated into the lumens. Several tumor foci showed secondary necrosis. The only metastases, found microscopically, were in the bronchial nodes. Malassez called the condition "encephaloid carcinoma" of the lung and was uncertain as to whether it was of alveolar or of bronchial origin. His excellent description, however, presents the essential features of the multiple nodular type of alveolar cell tumor.

*Gross Character.*—On the basis of Malassez' description and the literature [1] from that time to the present, the following morphologic picture is developed. There are two forms distinguishable grossly, the multiple nodular type and a diffuse form discovered in later years. In the former many tumor nodules are found more or less evenly distributed over both lungs. The lungs in many instances are greatly increased in weight, individual organs weighing as high as 2,360 Gm. and 2,750 Gm. (Briese). The nodules vary from miliary to marble size and are usually yellowish gray, pink or white, with fairly sharp borders. Most of the nodules are isolated, globular and of firm consistency; some may fuse to form larger masses. In others necrotic areas and mucinous secretion are commonly observed. The intervening lung tissue sometimes exhibits varying degrees of inflammation and compression, but it may be fairly intact. Tumor extension through the lymphatics is occasionally seen grossly as a network of fine strands spreading between the nodules. The visceral pleura may display protruding tumor nodules or more or less deep indentations which appear to be due to contraction by subpleural

1. This omits inaugural dissertations before 1900, which were published by candidates for the degree M.D. Such dissertations were especially common in Germany. Pässler in a comprehensive review of lung tumors in 1896 included practically all dissertations and cases up to that time. We refer the reader to his paper.

tumor foci. Some cases show unilateral or bilateral pleuritis with fibrinous or serous exudate, which may lead to compression of the lung. In later stages in such cases dense sheets of adhesions sometimes result. At autopsy the pathologist frequently gets the impression of metastatic cancer or miliary tuberculosis.

The other type of alveolar cell tumor, the diffuse form, was first described by Musser in 1903 and is rarer. It is characterized by involvement of a lobe or an entire lung on one or both sides. The tissue reaction grossly simulates lobar pneumonia in the stage of gray hepatization, and in some of the reported cases such a diagnosis was made on first examination. This picture has been presented in several reports. In other cases diffuse involvement of one side is accompanied by the presence of satellite nodules with or without nodular involvement on the other side.

The discussion of metastases applies to both the multiple nodular and the diffuse type. Metastases are not a prominent feature as a rule. In approximately half of the cases they were not found. An additional 25 per cent showed involvement of the hilar and bronchial lymph nodes only. In the remaining 25 per cent distant metastases were found, mainly in the liver, remote lymph nodes, kidneys, brain, adrenals and pericardium in this order of frequency. The gross appearance of the metastatic lesions is not characteristic.

*Microscopic Picture.*—The two forms of the tumor are identical histologically, and in many details the description that Malassez furnished is adequate. The tumor stroma is made up of the alveolar walls. The latter are lined by cuboid or columnar neoplastic cells in one or more layers. Papillary protrusions of such cells are frequent. In many instances the tumor cells become exfoliated into the lumens and assume a round and regular form. In other fields whole layers or sheets of lining cells may become detached from the wall.[2] Where this occurs in the presence of papillary formation, spherical nests of cells are seen in the alveolar lumens with an appearance under low magnification much like that of renal glomeruli. Occasionally, alveolar phagocytes may be seen closely related to the tumor cells. The nuclei of many of the latter are oval and pale, with prominent pink nucleoli; others, however, have hyperchromatic nuclei. Cilia are not found. Sometimes flattened, bizarre and giant cells are observed. Mitotic figures may be fairly frequent. Occasional tumor foci undergo secondary necrosis. In some

2. Personal experience has shown that the attachment of tumor cells to the alveolar wall is very delicate. Only careful embedding in paraffin or preferably celloidin will prevent many of the tumor cells from being detached and lost. In frozen sections, sometimes the entire tumor structure falls out, leaving the framework behind, intact.

TABLE 1.—*Cases of Alveolar Cell Tumor*

| Author | Age and Sex of Patient | Pathology | Author's Interpretation | Comment |
|---|---|---|---|---|
| Malassez..... 1876 | 47 F | Gross: bilateral nodular lung tumor. Microscopic: alveolar walls preserved and lined by cylindric tumor cells with papillary protrusions, cyst formation, mucus and necrotic foci. Metastases: bronchial lymph nodes | Epithelioma, but author undecided as to whether of bronchial or alveolar origin | Classic case of multiple nodular type of alveolar cell tumor, repeatedly cited in literature |
| Finlay and Parker... 1877 | 37 M | Gross: multiple nodular involvement of both lungs. Microscopic: cylindric epithelium lining alveolar walls; alveolar structure preserved. Metastases: bronchial lymph nodes and liver | Primary cancer of the lung | Multiple nodular type of alveolar cell tumor |
| Musser..... 1903 | 47 M | Gross: diffuse grayish yellow infiltration of both lungs. Microscopic: cuboid and columnar tumor cells forming papillae; alveolar structure preserved. Metastases: bronchial, tracheal, cervical and retroperitoneal lymph nodes | Alveolar cell cancer | Diffuse type of alveolar cell tumor |
| Kretschmer..... 1904 | 44 F | Gross: nodular, frequently coalescing foci of soft and mucinous consistency and of grayish white color. Microscopic: alveoli lined by cylindric and cuboid cells with papillary proliferation. Metastases: bronchial lymph nodes and pericardium | Alveolar cell cancer arising from alveolar epithelium and respiratory bronchioles | Multiple nodular type of alveolar cell tumor with coalescing foci |
| Pepere..... 1904 | 57 F | Gross: diffuse gray infiltration of both lungs similar to gray hepatization of pneumonia. Microscopic: cylindric cell tumor arising in alveolar epithelium and in epithelium of smallest bronchioles. Metastases: brain | Rare type of cancer arising from alveolar and respiratory bronchiolar epithelium | Diffuse type of alveolar cell tumor |
| Helly..... 1907 | 43 F | Gross: multiple nodular bilateral tumor. Microscopic: high cylindric epithelium lining alveoli. Metastases: none | Adenoma rather than carcinoma because of absence of metastases or lymphatic involvement and preservation of alveolar walls | Multiple nodular type of alveolar cell tumor |
| Knierim..... 1909 | 59 F | Gross: diffuse tumor with satellite nodules localized to right lung. Microscopic: alveolar cell proliferation of cuboid and cylindric types with papillae; mucinous masses; polyhedral cells in dense accumulations. Metastases: hilar, retroperitoneal and retrogastric lymph nodes | Primary pulmonary cancer originating in alveolar and respiratory bronchiolar epithelium | Alveolar cell tumor with mixed diffuse and nodular features |
| Briese..... 1920 | 49 M | Gross: multiple nodular tumor, bilateral. Microscopic: alveolar structure preserved; walls lined by high cylindric or cuboid cells with papillary projections. Metastases: prevertebral and bronchial lymph nodes | Papillary, gelatinous adenocarcinoma arising in alveolar epithelium | Multiple nodular type of alveolar cell tumor |
| Gordon..... 1920 | 56 F | Gross: right lung infiltrated by homogeneous massive growth; looked like gray hepatization. Microscopic: alveolar structure preserved and lined by columnar epithelial cells. Metastases: none | Primary diffuse alveolar cancer | Diffuse type of alveolar cell tumor in right lung |
| Schmincke..... 1922 | .. .. | Gross: two cases of diffuse tumor infiltration of both lungs; no primary focus elsewhere. Microscopic: intraalveolar and intravascular growth; in part solid and in part adenomatous; mucin produced in some fields. Metastases: none | Diffuse primary carcinoma of lung in both cases ("holoblastosis") | Diffuse forms of alveolar cell tumor |
| Gödel..... 1923 | 20 M | Gross: bilateral tumor involvement; diffuse grayish white infiltration of upper lobes; nodular in lower and middle lobes. Microscopic: alveoli lined by cuboid or low cylindric cells with papillary formations; mucus production. Metastases: hilar lymph nodes | Alveolar epithelial cancer | Alveolar cell tumor with mixed diffuse and nodular features |

| | | | | |
|---|---|---|---|---|
| Hedinger, 1923 | 32 M | Gross: bilateral tumor involvement; on the left, like caseous pneumonia; on the right, like miliary carcinosis. Microscopic: alveoli filled with tumor cells and septums preserved; lymphatics and smaller vessels contain tumor cells. Metastases: none | Primary diffuse pulmonary cancer arising from alveolar epithelium | Alveolar cell tumor with mixed diffuse and nodular features |
| Hedinger, 1923 | 89 F | Gross: bilateral diffuse gray hepatization. Microscopic: alveolar lumens filled with glandular structures and cylindric cells. Metastases: left kidney, spleen, liver | Primary diffuse cancer of lung arising from alveolar epithelium | Diffuse type of alveolar cell tumor |
| Rusk and Randolph, 1924 | 45 F | Gross: both lungs diffusely involved by apparent gelatinous and caseous pneumonia. Microscopic: normal alveoli lined by several layers of cuboid or low cylindric epithelial cells. Metastases: mediastinal lymph nodes, kidneys, liver, urinary bladder | Primary cancer of lung | Diffuse type of alveolar cell tumor |
| Fried (b) (also Hyde Holmes), 1925 | 52 M | Gross: left lung, multiple nodular tumor infiltration; right, mixed consolidation and nodular invasion. Microscopic: tumor confined to alveoli; normal lining replaced by cuboid or columnar tumor cells. Metastases: liver, rib, left lung | Primary carcinoma of right lung, probably of alveolar origin | Alveolar cell tumor with mixed diffuse and nodular features |
| Reuss, 1934 | 46 M | Gross: bilateral nodular foci, partly coalescing, with a mucinous appearance. Microscopic: cylindric cells filling the alveoli; elastic framework preserved. Metastases: none | Multicentric alveolar carcinoma | Multiple nodular type of alveolar cell tumor |
| Reuss, 1934 | 74 M | Gross: multicentric gelatinous foci in all lobes, both lungs; deep surface indentations. Microscopic: high cylindric epithelial cells growing in alveoli; gelatinous masses in lumens of alveoli. Metastases: hilar lymph nodes | Multicentric alveolar carcinoma | Multiple nodular type of alveolar cell tumor |
| Sweany, 1935 | 47 F | Gross: on right, uniform pneumonia-like consolidation; on left, same in midportion and scattered nodules elsewhere. Microscopic: alveoli lined by low columnar, nonciliated cells with papillary projections; alveolar walls preserved. Metastases: hilar lymph nodes | Origin somewhere between lower respiratory bronchioles and alveoli; author designates it as "so-called alveolar cell carcinoma" | Description conforms to predominantly diffuse type of alveolar cell tumor |
| Weissmann, 1935 | 63 M | Gross: lungs small and nodular; on section multiple grayish white foci. Microscopic: alveolar structure preserved and lined by cuboid or columnar nonciliated cells; no mucus production; septums thickened owing to proliferation of elastic fibers. Metastases: none | Diffuse primary alveolar epithelial carcinoma | Multiple nodular type of alveolar cell tumor |
| Weissmann, 1935 | 65 F | Gross: mixed, grayish white, diffuse infiltration and, in addition, solitary or coalescing tumor nodules. Microscopic: similar to previous case but with production of mucus. Metastases: hilar lymph nodes | Diffuse primary alveolar epithelial carcinoma | Predominantly multiple nodular type of alveolar cell tumor |
| Graber, 1938 | 49 F | Gross: densely accumulated milary nodules in both lungs, similar to bronchopneumonic foci on section. Microscopic: alveoli lined by high cylindric epithelial cells in some places forming papillae; in others becoming desquamated. Metastases: brain | Primary multicentric cancer of lungs | Multiple nodular type of alveolar cell tumor |
| Bonne, 1939 | 30 M | Gross: bilateral diffuse homogeneous tumor; looked like pneumonic hepatization. Microscopic: alveoli lined by cuboid and columnar cells with papillary projection; no destruction of septums. Metastases: none | Carcinosis originating in terminal bronchioles and alveoli | Diffuse type of alveolar cell tumor |
| Casilli and White, 1940 | 57 M | Gross: all lobes involved by various-sized nodules; partly confluent. Microscopic: air sacs filled and distended with polygonal cells; interstitial framework spared. Metastases: peribronchial lymph nodes | Carcinomatoides alveogenica multicentrica | Multiple nodular type of alveolar cell tumor |
| Neubuerger, 1941 | 39 M | Gross: multiple nodules bilaterally. Microscopic: alveoli generally well preserved and lined by cuboid or cylindric cells with papillary growth. Metastases: mediastinal and periaortic lymph nodes; liver and kidneys | Primary multiple alveolar cell tumor | Multiple nodular type of alveolar cell tumor |

TABLE 2.—*Controversial Cases of Alveolar Cell Tumor*

| Author | Age and Sex of Patient | Pathology | Author's Interpretation | Comment |
|---|---|---|---|---|
| Renant..... 1876 | 60 M | Gross: solitary caseous tumor the size of a fist in middle of right lung. Microscopic: alveoli preserved and lined by polyhedral or cylindric cells in periphery of tumor; central necrosis of tumor cells, but alveolar walls still preserved. Metastases: none grossly, but lymphatics involved by tumor cells microscopically | No specific diagnosis except unusual tumor of lung. Malassez believed this case similar to his own | Strong possibility of early stage of diffuse alveolar cell tumor |
| Siegert..... 1883 | 53 F | Gross: diffuse pneumonia-like consolidation in central areas of both lobes of left lung; right uninvolved. Microscopic: alveolar epithelium replaced by large cuboid or columnar cells arising from alveolar walls (in some places flattened and similar to squamous epithelium); cancerous lymphangitis. Metastases: peribronchial lymph nodes | Epithelial cancer of lung arising from alveoli | Has features of diffuse type of alveolar cell tumor, but presence of squamous epithelium makes diagnosis doubtful |
| Pässler..... 1896 | 46 F | Gross: multiple nodular involvement of left lung; right lung presented a solitary node. Microscopic: tumor cells lining intact alveolar walls in a single layer with papillary projections. Metastases: none | Primary cancer of doubtful origin; believes pulmonary tumors essentially bronchogenic | Probable alveolar cell tumor of multiple nodular type affecting mainly left lung |
| Edlavitch..... 1912 | 50 M | Gross: middle and lower lobes of right lobe were solid and on section opaque and dull gray; other lung uninvolved. Microscopic: alveoli filled with desquamated epithelium; tumor cells with a definite alveolar pattern, some of squamous type. Metastases: hilar nodes | Primary pulmonary cancer of alveolar origin | Some features of similarity to diffuse type of alveolar cell tumor; presence of squamous epithelium, however, makes origin doubtful |
| Elsmayer..... 1924 | 55 F | Gross: bilateral nodular tumor involvement of grayish white or yellow color; mucinous fluid. Microscopic: alveoli lined by cylindric and cuboid cells with papillary formation; vacuoles in cells and masses give positive mucicarmine reaction. Metastases: hilar, subclavicular, right carotid and peripancreatic lymph nodes | Primary gelatinous carcinoma of lung, source undetermined; does not believe in multiple alveolar origin | Strong possibility of multiple nodular type of alveolar cell tumor |
| Schuster..... 1929 | 44 M | Gross: diffuse gelatinous tumor infiltration in both lungs. Microscopic: alveoli lined by columnar cells. Metastases: none | Primary tumor of lungs of undetermined origin; "more of pious hope than demonstration of fact" in writings of authors believing in alveolar carcinoma | Strong possibility of diffuse type of alveolar cell tumor |

| | | | | |
|---|---|---|---|---|
| Hamman..... 1933 | 57 F | Gross: bilateral and diffuse miliary infiltration. Microscopic: no microscopic description furnished. Metastases: peribronchial, peritracheal and cervical lymph nodes; spleen, kidneys, epicardium | Primary adenocarcinoma with diffuse miliary infiltration | Possibility of multiple nodular type of alveolar cell tumor but pathologic description incomplete |
| Lastra, Caeiro and Warcalde 1935 | 50 M | Gross: hepatization of right lower lobe with cavitation; lobular lesions in other lobes and in left lung. Microscopic: columnar cells lining alveoli and forming papillary projections. Metastases: none | Diffuse pulmonary cancer originating in bronchial epithelium | Strong possibility of mixed diffuse and nodular alveolar cell tumor since bronchial origin unproved |
| Lindberg..... 1935 | 49 M | Gross: bilateral nodular carcinoma. Microscopic: areas of cancerous pneumonia; alveolar septums form tumor stroma. Metastases: none | Bronchogenic carcinoma; thickening of bronchus described without clarifying | Possible multiple nodular type of alveolar cell tumor |
| Frissel and Knox.... 1937 | 64 M | Gross: bilateral miliary carcinoma. Microscopic: alveolar walls preserved and lined by tall columnar cells with papillary arrangement and mucus secretion | Bronchogenic origin with primary site lost in mass of miliary infiltration | Strong possibility of multiple nodular type of alveolar cell tumor |
| Marshall..... 1937 | 38 M | Gross: pneumonia-like infiltration of right upper lobe. Microscopic: irregular tumor cells filling alveoli. Metastases: brain, left adrenal | Alveolar cancer with metastases | Has features of diffuse type of alveolar cell tumor but microscopic description inconclusive |
| Uspensky..... 1937 | 28 F | Gross: solitary nodule, size of hen's egg in left lung. Microscopic: author says typical alveolar cancer but furnishes no description. Metastases: brain | Alveolar cell carcinoma; emphasizes the duration of 8 yr. | Gross appearance not typical and microscopic description insufficient |
| Schäfer..... 1939 | 50 M | Gross: bilateral tumor in and about multiple lung cysts. Microscopic: 3 different patterns (a) high cylindric mucus-producing adenocarcinoma with preserved alveolar structure; (b) adenocarcinoma with partly preserved alveolar structures; (c) carcinoma simplex with polymorphous cells and loss of alveolar structure. Metastases: none | Multicentric origin of carcinoma in a cystic lung | Histologic features suggest some relation to alveolar cell tumor |
| Watanabe..... 1939 | 65 M | Gross: diffuse bilateral infiltration of solid gray to yellow color; some intervening normal zones; others of gangrene within the tumor. Microscopic: well preserved alveoli lined by polygonal and giant cells; also by cuboid and cylindric cells wtih papillary projection; similar cellular features in bronchioles and epithelial metaplasia in larger bronchi. Metastases: hilar, perigastric and retroperitoneal lymph nodes; liver, left adrenal, brain, pituitary | Diffuse bronchogenic carcinoma of lung arising in gangrenous cavity which was believed to be the oldest focus | Photomicrographs fail to give definite proof of bronchogenic origin; diffuse form of alveolar cell tumor cannot be excluded |
| Casilli and White.... 1940 | 58 M | Gross: bilateral evenly distributed nodular tumor. Microscopic: irregular papillary adenomatous structures lined by cuboid cells. Metastases: none | Malignant adenoma arising from the alveolar ducts | Gross features similar to multiple nodular type of alveolar cell tumor; histologic features are not quite consistent |

fields an isolated alveolus distant from the tumor is involved microscopically and is lined by neoplastic cells. Mucinous secretion is sometimes noted.

The stroma is composed of the alveolar walls. The latter may exhibit chronic inflammatory changes and sometimes may become markedly thickened. Elastic fiber stains frequently reveal an increase of the elastic framework. A hyperplastic stroma occasionally compresses the tumor parenchyma; thus the tumorous alveoli appear distorted and the lining cells flattened. Scarlike shrinkage of such nodules may follow. In other areas dilated tumorous alveoli may fuse because of a breakdown of the walls, to form larger sacs simulating somewhat ordinary adenocarcinoma. This is rare, however, and the alveolar pattern is definitely the prevailing one. The lymphatics have often been found to contain tumor cells. Chronic pneumonia, sclerosis, atelectasis and emphysema are not infrequently noted in the intervening lung tissue. In most cases of alveolar cell tumor which have been examined carefully,[3] no primary bronchiolar involvement was found microscopically. In a few instances neoplastic changes had occurred in the bronchiolar mucosa, but the alveolar proliferation appeared to be independent. Rarely, bronchial erosion by tumor from without was observed. The metastatic lesions are essentially similar to the primary tumor, but the pattern is not quite as regular, and the picture may resemble adenocarcinoma.

We have arranged in the form of tables all cases encountered in the obtainable literature, in which the features conformed with those described here. The cases are listed chronologically and offer an easy survey to the reader. In the first table, listing 25 cases, we have included only those with unmistakable evidence grossly and microscopically of the alveolar cell tumor. The authors reporting the cases in this group either made the diagnosis of a tumor arising entirely or at least in part from the alveoli, or admitted the possibility of this.

In the second table (15 cases) we present controversial material. Some authors reported tumors with many or all the features of the alveolar cell tumor but discarded such an origin and made a diagnosis of bronchogenic tumor. However, they were not able to demonstrate the primary site satisfactorily. We have included in this table several cases in which the diagnosis of a tumor arising from the alveoli has been made on insufficient or objectionable evidence.

The third table contains only a few cases in which primary lung tumors found incidentally at autopsy had some characteristics common to the alveolar cell tumor and the changes can possibly be considered as precancerous.

3. Six cases have been studied thoroughly by us.

TABLE 3.—*Primary Pulmonary Tumors with Some Characteristics of Alveolar Cell Tumor*

| Author | Age and Sex of Patient | Pathology | Author's Interpretation | Comment |
|---|---|---|---|---|
| Björksten..... 1904 | 9 M | Gross: patient died of scarlet fever; white tumor nodules in both lungs. Microscopic: small cysts lined by cylindric epithelium with papillary projections. Metastases: none | Papillary cystadenoma arising from alveolar epithelium | Gross appearance similar to multiple nodular type of alveolar cell tumor; this case is, however, benign |
| Löhlein..... 1908 | 69 F | Gross: patient died of tuberculous pericarditis; right lower lobe occupied by firm tumor containing thick, jelly-like mucus in cavities of various size. Microscopic: alveoli cystic and lined by high cylindric epithelial cells with papillary protrusions, the lumens being filled with mucus. Metastases: none | Cystic papillary tumor | Benign cystic tumor related in both gross and microscopic features to diffuse type of alveolar cell tumor |
| Oberndorfer..... 1930 | 21 M | Gross: patient died of septic pneumonia; miliary yellowish nodules bilaterally distributed through pneumonic consolidation. Microscopic: in yellow nodules, alveoli lined by high cuboid epithelium with papillary protrusions and desquamation; typical hemorrhagic pneumonia in background. Metastases: none | Precancerous mutation of alveolar epithelium under the influence of hemorrhagic septic pneumonia | Premalignant stage of multiple nodular type of alveolar cell tumor may be a possibility in this case |
| Richardson..... 1940 | 73 F | Gross: patient died of biliary obstruction; lungs showed scattered yellow gelatinous nodules. Microscopic: alveoli lined by tall columnar epithelium secreting thick mucus. Metastases: none | Mucus-producing adenomatosis of bronchogenic origin; author believes in a downgrowth of bronchiolar epithelium | Possible premalignant stage of multiple nodular type of alveolar cell tumor |

## INCIDENCE

On the basis of the reports submitted thus far the reader might dismiss the whole subject as one of a curious and rare condition. The alveolar cell tumor is, however, given lengthy consideration in standard textbooks (Ewing; Kaufmann; Letulle) and in W. Fischer's article in the Henke-Lubarsch handbook, and one obtains the impression that these authors have had personal experience with it. Oberndorfer stated in 1933 that he had seen 4 cases of this type and described the pathologic features but furnished no formal report. O. Klotz, in 1927, mentioned 1 instance of multiple nodular tumor in a series of 24 cases of pulmonary cancer. And in 1938 M. Klotz, reviewing primary carcinoma of the lung, described 2 personal observations of the diffuse type. However, complete details were not given and such instances could not be included in the charts. One of us (K. N.) has performed autopsy in 3 cases in former years in Germany and has reported a fourth. In this department of pathology we have seen one alveolar cell tumor of the diffuse and another of the nodular type, both cases unpublished, within the past year.

Since the number of undebatable instances of this tumor is so small, satisfactory statistical data as to its incidence relative to that of other pulmonary tumors are meager. Reuss, however, stated that he found 2 such tumors among 46 pulmonary tumors, and Oberndorfer in 1933 had observed 4 among 150 pulmonary neoplasms.

Most statistics on carcinoma of the lung show a predominance in males of 3:1 (Egenolf) or 4:1 (Fischer-Wasels). In the cases of definitely established alveolar cell tumor listed in table 1, the proportion is different. Age and sex were not given in the 2 cases of Schmincke, but the remaining 23 showed a distribution of 12 males and 11 females, or approximately a 1:1 ratio. The ages varied from 20 to 89 years with the chief incidence, in 14 cases out of 23, between 40 and 60 years.

## GENESIS

If we assume that this tumor arises from the walls of the pulmonary alveoli, then we should devote our attention briefly to a consideration of the alveolar structure as it exists normally and under various pathologic conditions. Since the literature is immense, we shall present only material that aids in the understanding of the alveolar cell tumor.

*Normal Structure of the Alveoli.*—The nature of the cells bordering the alveoli in normal adult life is highly controversial. At present, three well defined views exist: first, that the alveoli have a continuous epithelial lining (Miller; Bremer, and others); second, that the alveoli have a discontinuous epithelial lining in the form of isolated cells or nests in the niches between the capillaries (Aschoff; Seemann; Bargmann); third, that no true lining exists but that the capillaries are contained in a

ground substance with occasional "septal cells," probably of mesenchymal origin (Lang; Bloom; Loosli). The third view appears to be gaining more and more advocates, particularly in this country. We refer the interested reader to the round table conference conducted by Macklin and to the comprehensive and recent paper of Loosli.

*Alveolar Lining Under Pathologic Conditions.*—It is universally agreed by all workers that under certain pathologic conditions the alveoli can demonstrate a definite lining. The cells of this lining are cuboid or columnar and may become detached from the wall individually or in continuous rows. Such changes are observed under a variety of conditions described in textbooks (MacCallum) and in many papers (Brandt; Graef; Dawson; Wohlwill). For example, chronic pulmonary disorders, as silicosis or tuberculosis, may demonstrate such zones of proliferation in or about the margins of the diseased areas. Atelectatic zones sometimes exhibit them. In more acute form, they may appear in proliferative interstitial pneumonia with predominantly mononuclear exudate, as is found notably in virus infections, influenza and psittacosis being characteristic. The proliferation of alveolar lining cells may become so prominent in the course of the conditions mentioned that it resembles histologically the picture found in an alveolar cell tumor.

*Experimental and Spontaneous Changes in Animals.*—Experimental evidence is abundant as to the proliferation of lining cells in the pulmonary alveoli of animals. Such changes have been brought about by various chemicals, oils, gases and toxins (Young; Winternitz, Smith and McNamara; Seelig and Benignus; Simonds and Curtis). Ross recently reviewed this question. More important perhaps in relation to our main subject are the experimental and spontaneous tumors believed to arise in the alveolar walls. Such tumors can be produced in mice by the subcutaneous injection of certain aromatic hydrocarbons (Grady and Stewart). The tumors are multicentric and histologically appear to arise in the alveolar walls. Spontaneous pulmonary neoplasms in the same animal have been described in a great number of instances, particularly by Slye, Holmes and Wells. The histologic evidence of alveolar origin appears well established in their report.

We should also like to refer briefly to an interesting contagious pulmonary disease occurring particularly in sheep and designated under a variety of terms, as "Montana chronic progressive pneumonia," "jagziekte" and "epizootic adenomatosis" (Cowdry and Marsh; Dungal). In this condition multiple nodular or diffuse tumor-like foci are found within areas of chronic pneumonia. The bronchi are not involved. Microscopic examination of the nodule shows a proliferation of alveolar cell lining resembling the alveolar cell tumor. To a much less degree the bronchiolar epithelium also shows evidence of stimulation. The

chronic interstitial inflammation found in the background is believed to be the decisive causative factor. Bonne and others have directed attention to the relation of this interesting condition to the tumor under discussion in man.

*The Alveolar Cell Tumor.*—Before discussing histogenesis, we should like to consider the problem of primary multiplicity. The older conception concerning tumor origin embraced the belief that every neoplasm develops from a single and circumscribed anlage and then extends from that point. However, there is support for the assumption of multiple origin in certain well established tumors, for example, diffuse hemangioendothelioma of the liver and multiple carcinoma occurring in cirrhosis of the same organ. Multiple myeloma of the skeletal system and the multiple foci of origin sometimes demonstrated in lymphosarcoma are further examples. Moreover, several investigators (Feyrter; Lindberg) observed that in bronchogenic carcinoma minute primary cancerous foci may be seen microscopically in many bronchial branches. Furthermore, Kraft in his paper furnished an excellent photograph of a gross specimen of primary multiple bronchogenic carcinoma. To express the idea of even more extensive tumor origin, affecting the entire organ from the very beginning of growth, Schmincke introduced the term "holoblastosis."

Most authors who have believed in the existence of the alveolar cell tumor have felt that it arises from multiple primary foci (the nodular type) or more extensively in large pulmonary areas (the diffuse form). However, those authors who reported the cases listed in table 2 have believed differently (Eismayer; Lindberg, and Frissell and Knox among others). According to these authors, the so-called alveolar cell tumor is in reality a bronchogenic tumor arising from some single focus and then metastasizing rapidly to other parts of the lungs. A demonstration of the primary focus, however, has been impossible. Its absence has been explained by such statements as that of Frissell and Knox: "The origin was in some small area, which could not be distinguished from the mass of miliary infiltration." Opponents of the existence of the alveolar cell tumor go on from this point to explain the characteristic histologic pattern on the basis of epithelial tumor downgrowth from the bronchial tree, extension through the pores of Kohn and lymphatic or vascular extension or both. In each instance subsequent intra-alveolar growth results, according to this interpretation. The idea that the tumor arises in multiple foci from the alveoli seems to us preferable because of the failure to show a primary bronchial focus and because no tumor downgrowth or extension through the pores of Kohn was observed in the cases that we have had an opportunity to study. Lymphatic and vascular extension cannot be denied, but it is uncertain whether such extension or rupture of the lymphatics (Lindberg) can reproduce the histologic appearance of alveolar cell tumor in other parts of the lungs.

The authors who believe in an alveolar origin find the histogenesis burdened with a special problem in addition to the problems that are associated with all neoplasms. This is due to the controversy concerning the character and the origin of the alveolar lining, as mentioned in an earlier paragraph. The workers who believe in the presence of an epithelial alveolar lining in the adult lung readily accept the tumor and trace it to this epithelium. They designate it as carcinoma (see table 1). In this they are in contrast with those who deny the existence of an alveolar epithelial lining and on that basis discard the possibility of alveolar cell tumors. Arkin and Wagner, for example, stated: "There can be no carcinoma primary in the alveoli as they have no epithelial lining." Fried,[4] in 1931, and Geschickter and Denison, in 1934, appeared to entertain essentially the same belief and objected to the tumor on this basis. Neubuerger attempted to reconcile the two positions, namely, the existence of a characteristic alveolar cell tumor and the absence of alveolar epithelial lining. He considered that the alveolar lining as seen under pathologic conditions develops from mesenchymal septal cells and that a continuous developmental relation exists between the occasional production of lining in inflammatory conditions, on the one hand, and the alveolar cell tumor, on the other. He stressed the similarity of the histogenesis of this tumor to that of the experimental neoplasms described by Grady and Stewart. However, the difficulty of tracing tumors with carcinoma-like histologic features to a mesenchymal origin was emphasized, and no decision was reached at that time. Since that report, a study by J. Oppenheimer has come to our attention which appears to lessen the importance of this question. She summarized the history and the views concerning the specificity of the germ layers and concluded from a review of all important work in this field: "The doctrine of the absolute specificity of the germ layers as enunciated in the last century must be abandoned." She also quoted Brachet as stating: "In reality the germ layers like the blastomeres have an actual potentiality and a total potentiality; the former is what they normally become; the latter what they are capable of forming in addition under diverse natural or experimental influences." Thus the possibility of mesenchymal cells giving rise to tumors with epithelial-like cellular elements cannot at present be rejected.

## ETIOLOGY

The causes of alveolar cell tumor are as obscure as those of many other forms of neoplasm. One of the significant possibilities mentioned in the cases we have presented is that of a virus origin as suggested by

4. Fried apparently changed his opinion after 1925. In that year he reported the case listed in table 1, and at that time his diagnosis was: "Primary carcinoma of the right lung probably of alveolar origin with metastases to the left lung, pleura, rib and liver."

Bonne. He compared the etiologic evidence in his case to that of "jagziekte," the disease in sheep we mentioned. The suggestion is interesting but is as yet unproved. The condition reported by Oberndorfer in 1930, which he considered precancerous, is also of interest. The tumor-like structures probably developed in the course of pneumonia, and this points to the possibility that inflammatory conditions which stimulate proliferation of alveolar lining may, in rare and extreme cases, be responsible for malignant degeneration. It has not been demonstrated that such definite factors as radioactive substances and chromic or tar compounds, which appear significant in relation to bronchogenic tumors, play a role in the alveolar cell tumor occurring in man. However, alveolar cell tumors have been produced in mice by the injection of various chemicals (Grady and Stewart, and others), and although such results cannot be directly applied to man, this experimental field offers hope for future clarification of etiologic problems.

### DIFFERENTIATION FROM OTHER PULMONARY TUMORS

*Bronchogenic Tumors.*—Only careful gross and microscopic examinations together will enable the pathologist to diagnose the alveolar cell tumor. Interpretations based on gross or histologic observations alone may be misleading, since other tumors simulate the alveolar cell neoplasm in one feature or another. Metastatic or bronchogenic carcinoma, for example, may use the alveolar structure as a stroma for the tumor extension (Stahr; O. Klotz). Among the bronchogenic tumors we can readily dismiss from consideration squamous, basal cell and undifferentiated (e. g., "oat cell") carcinoma, as a combined gross and microscopic study of these types offers easy differentiation. Adenocarcinoma arising from the mucinous glands or from the bronchial mucosa may cause greater difficulty in differential diagnosis, particularly if the gross bronchial involvement is not clearly recognizable. The microscopic picture, however, is characteristic. In the tumor arising from mucinous glands, the involvement of the submucosa and the similarity of the tumor acini to mucinous glands, features emphasized by Ewing, and finally the absence of uniform, preserved alveoli lined by tumor cells assist in the differentiation. The presence or absence of mucus in itself is not pathognomonic, for not only the specific glandular cells but also other types are capable of such production under certain circumstances (Ernst). Adenocarcinoma arising from the bronchial mucosa is of even greater importance in the differential diagnosis from the alveolar cell tumor. Opponents of the existence of the latter base their objections on the apparent lack of microscopic distinction. Bronchogenic adenocarcinoma may resemble certain areas of alveolar cell tumor showing breakdown of the alveolar septums. However, examination of larger areas will

demonstrate that irregular adenomatous structures predominate and tumor growth in preserved alveoli is infrequent in adenocarcinoma, whereas the reverse ratio is true of the alveolar cell tumor. It must be admitted that occasional instances are encountered in which absolute differentiation may be difficult microscopically. The mixed tumors, as described by Womack and Graham, are usually benign, definitely bronchogenic neoplasms; they will hardly cause confusion, although occasionally structures resembling lined fetal lung tissue (and thus alveolar cell tumor) may be observed.

*Metastatic Tumors.*—Of the two types of alveolar cell tumor, only the multiple nodular form could be confused with a new growth arising in another part of the body and metastasizing to the lungs. Therefore, a primary neoplasm elsewhere must be carefully ruled out. This requirement appears to have been satisfied by a thorough postmortem examination in the cases in table 1. It is well known that various primary tumors—for example, those of the breast, thyroid, genitourinary tract and bones—metastasize in a multiple nodular form to the lungs. Nicholson described an ovarian tumor with distant metastasis exclusively to the lungs, the latter being studded with multiple nodules which microscopically were strikingly similar to those observed in the alveolar cell tumor. Pick commented on this case, demonstrated the similarity of the tumor to the one described by Helly and emphasized the need of caution in the diagnosis of primary multiple nodular pulmonary neoplasm. However, the combination of circumstances described by Nicholson appears to be unique.

## CLINICAL FEATURES

*Symptoms.*—A study of the clinical records in the cases presented in table 1 reveals no distinctive symptoms that would enable one to differentiate this condition from other pulmonary tumors. In some instances the symptoms were prolonged over several months, and the diagnosis of pulmonary tuberculosis was made. In other cases the course was rapid, and the impression was one of lobar or bronchial pneumonia. The majority of the patients did not survive more than a year, and usually the duration of life was much less. The course was only three to four weeks in Hedinger's cases. On summarizing the available clinical data of all cases, one obtains the impression that the duration of life is less than with other forms of untreated pulmonary malignant neoplasms. The symptoms are similar to those encountered in bronchogenic tumors, namely, cough, bloody sputum, cyanosis, pain in the chest and evidence of pleural effusion. Occasional reports describe the prevailing symptoms as due to tumor metastasis, for example, in the brain.

*Diagnosis.*—In the published reports one encounters instances in which the diagnosis of tumor of the lung was established clinically, par-

ticularly when the course was protracted. But in no case was a differential diagnosis of alveolar cell tumor made. Roentgenologic and bronchoscopic studies are of major importance in both direct and differential diagnosis. The roentgen examination furnishes proof of the presence of a multiple nodular tumor of the lung. From that point, further studies may be undertaken to determine whether the lesions are metastatic, and when this possibility can be excluded, the presence of a multiple nodular alveolar cell tumor should be considered. The only other diseases that might confuse one roentgenologically are miliary tuberculosis and silicosis, but the clinical features are so distinct that differentiation is usually not difficult.

If the roentgen examination and clinical picture suggest a diffuse pulmonary neoplasm, bronchoscopic study should aid in the diagnosis. If such a study demonstrates no involvement of the bronchial tree as far as it can be examined in this manner, then the possibility of the diffuse form of the alveolar cell tumor should be considered. It is true, however, that among the cases of the diffuse type listed in table 1 were several in which the course was very short—only a few weeks or months; in such instances the differentiation from massive pneumonia, rapidly advancing tuberculosis and rarer conditions, such as fungus infections, was apparently impossible.

## PROGNOSIS AND TREATMENT

The outlook for the patient suffering from alveolar cell tumor is poor. Of the two forms, it would appear that the diffuse offers a slightly more favorable prognosis if diagnosed while it is localized to one side. In such instances lobectomy or pneumonectomy may be indicated, especially since metastases are not prominent. Roentgen therapy also appears applicable as a palliative measure in this form of the alveolar cell tumor. The multiple nodular form would not appear to be amenable to roentgen treatment for obvious reasons, and because of the frequency of bilateral involvement, surgical intervention is impossible.

## SUMMARY

The bulk of evidence submitted thus far points to the existence of a specific tumor of the lung arising from and lining the alveolar walls. Many authors call this neoplasm carcinoma. The term "alveolar cell tumor" appears preferable because of the uncertainty as to the histogenesis and because the trend of present opinion seems to favor the idea that the alveolus of the adult lung is not lined by epithelium.

This tumor arises as a multiple nodular neoplasm or, less commonly, as a diffuse or lobar infiltrating growth; combinations of both forms are occasionally observed.

A survey of the literature provides a number of controversial cases in which the authors make the diagnosis on insufficient grounds or in which the possibility of an alveolar cell tumor is rejected. A study of some of the latter reports would appear to indicate the presence of an alveolar cell tumor. In others no decision could be made on the basis of the evidence submitted.

The tumor appears quite uncommon as cases are gathered from the literature. Certainly, the incidence is no more than 5 per cent of pulmonary new growths. On the basis of personal experience, however, the alveolar cell tumor does not seem to be as rare as heretofore believed. It is anticipated that as more careful thought and examination are given to this subject by pathologists, roentgenologists and clinicians, the number of cases will increase in the future.

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## BIBLIOGRAPHY

Arkin, A., and Wagner, D. H.: J. A. M. A. **106**:587, 1936.

Aschoff, L.: Frankfurt. Ztschr. f. Path. **48**:449, 1935.

Bargmann, W.: Die Lungenalveole, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1936, vol. 5, pt. 3, pp. 821-834.

Björksten, M.: Finska läk.-sällsk. handl. **46**:9, 1904.

Bloom, W., in Maximow, A. A., and Bloom, W.: Textbook of Histology, ed. 3, Philadelphia, W. B. Saunders Company, 1938, pp. 456-459.

Bonne, C.: Am. J. Cancer **35**:491, 1939.

Brandt, M.: Virchows Arch. f. path. Anat. **262**:211, 1926.

Bremer, J. L.: Contrib. Embryol. **25**:83, 1935.

Briese: Frankfurt. Ztschr. f. Path. **23**:48, 1920.

Casilli, A. R., and White, H. J.: Am. J. Clin. Path. **10**:623, 1940.

Cowdry, E. V., and Marsh, H.: J. Exper. Med. **45**:571, 1927.

D'Aunoy, R.; Pearson, B., and Halpert, B.: Am. J. Path. **15**:567, 1939.

Dawson, J.: J. Path. & Bact. **49**:483, 1939.

Dungal, N.: Proc. Roy. Soc. Med. **31**:497, 1938.

Edlavitch, B. M.: J. A. M. A. **59**:181, 1912.

Egenolf, W.: Ztschr. f. Krebsforsch. **31**:396, 1930.

Eismayer, G.: Ztschr. f. Krebsforsch. **21**:203, 1924.

Ernst, P.: Die Pathologie der Zelle, in Krehl, L., and Marchand, F.: Handbuch der allgemeinen Pathologie, Leipzig, S. Hirzel, 1915, vol. 3, pt. 1, p. 259.

Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, pp. 878-882.

Feyrter, F.: Wien. klin. Wchnschr. **40**:648, 1927.

Finlay, D., and Parker, R. W.: Lancet **1**:838, 1877.

Fischer, W., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1931, vol. 3, pt. 3, pp. 534, 535, 548 and 556.

Fischer-Wasels, B.: Acta d. internat. Vereinigung f. Krebsbekaempf. **3**:140, 1938.

Fried, B. M.: (*a*) Medicine **10**:373, 1931; (*b*) Arch. Int. Med. **35**:1, 1925.

Frissell, L. F., and Knox, L. C.: Am. J. Cancer **30**:219, 1937.

Geschickter, C. F., and Denison, R.: Am. J. Cancer **22**:854, 1934.
Gödel, A.: Frankfurt. Ztschr. f. Path. **29**:392, 1923.
Gordon, A. K.: Lancet **2**:501, 1920.
Graber, K.: Beitrag zur Histopathologie des primären Lungenkrebses, Inaug. Dissert., Munich, 1938; abstracted, Centralbl. f. Path. **77**:93, 1941.
Grady, H. G., and Stewart, H. L.: Am. J. Path. **16**:417, 1940.
Graef, I.: Arch. Path. **28**:613, 1939.
Hamman, L.: Am. Rev. Tuberc. **28**:711, 1933.
Hedinger, E.: Schweiz. med. Wchnschr. **53**:165, 1923.
Helly, K.: Ztschr. f. Heilk. **28**:105, 1907.
Hyde, T. L., and Holmes, G. W.: Am. J. Roentgenol. **18**:235, 1927.
Kaufmann, E.: Lehrbuch der speciellen pathologischen Anatomie, Berlin, Walter de Gruyter, 1931, vol. 1, p. 441.
Klotz, M.: Am. J. M. Sc. **196**:436, 1938.
Klotz, O.: Canad. M. A. J. **17**:989, 1927.
Knierim, H.: Verhandl. d. deutsch. path. Gesellsch. **13**:407, 1909.
Kraft, I. A.: Ztschr. f. Krebsforsch. **41**:51, 1935.
Kretschmer, W. H.: Ueber das primäre Bronchial- und Lungen-carcinom, Inaug. Dissert., Leipzig, 1904.
Lang, F. J.: J. Infect. Dis. **37**:430, 1925.
de Villafañe Lastra, T.; Caeiro, A., and Warcalde, J. G.: Prensa méd. argent. **22**:1477, 1935.
Letulle, M.: Anatomie pathologique, Paris, Masson & Cie, 1931, vol. 2, p. 901.
Lindberg, K.: Arb. a. d. path. Inst. d. Univ. Helsingfors **9**:1, 1935.
Löhlein, M.: Verhandl. d. deutsch. path. Gesellsch. **12**:111, 1908.
Loosli, C. G.: Am. J. Anat. **62**:375, 1938.
MacCallum, W. G.: A Textbook of Pathology, ed. 7, Philadelphia, W. B. Saunders Company, 1940, p. 745.
Macklin, C.: J. Thoracic Surg. **6**:82, 1937.
Malassez, L.: Arch. de physiol. **3**:353, 1876.
Marshall, R.: Ulster M. J. **6**:142, 1937.
Miller, W. S.: The Lung, Springfield, Ill., Charles C. Thomas, Publisher, 1937, pp. 56-64.
Musser, J. H.: Univ. Pennsylvania M. Bull. **16**:289, 1903.
Neubuerger, K.: J. Thoracic Surg. **10**:557, 1941.
Nicholson, G. W.: Ztschr. f. Geburtsh. u. Gynäk. **64**:252, 1909.
Oberndorfer, S.: (*a*) München. med. Wchnschr. **80**:688, 1933; (*b*) Virchows Arch. f. path. Anat. **275**:728, 1930.
Oppenheimer, J.: Quart. Rev. Biol. **15**:1, 1940.
Pässler, H.: Virchows Arch. f. path. Anat. **145**:199, 1896.
Pepere, A.: Centralbl. f. Path. **15**:948, 1904.
Pick, L., in discussion on Nicholson.
Renant, J., cited by Malassez.
Reuss, H.: Ueber 2 Fälle multicentrisch entstandener Lungenkrebse, Inaug. Dissert., Hamburg, 1934.
Richardson, G. O.: J. Path. & Bact. **51**:297, 1940.
Ross, I. S.: Arch. Path. **27**:478, 1939.
Rusk, G. Y., and Randolph, V.: J. A. M. A. **82**:442, 1924.
Schäfer, A.: Frankfurt. Ztschr. f. Path. **53**:263, 1939.
Schmincke, A.: Centralbl. f. Path. **33**:18, 1922.
Schuster, N. H.: J. Path. & Bact. **32**:799, 1929.

Seelig, M. G., and Benignus, E. L.: Am. J. Cancer **34**:391, 1938.
Seemann, G.: Histobiologie der Lungenalveole, Jena, Gustav Fischer, 1931, pp. 66-73.
Siegert, F.: Virchows Arch. f. path. Anat. **134**:287, 1893.
Simonds, J. P., and Curtis, J. S.: Arch. Path. **19**:287, 1935.
Slye, M.; Holmes, H. F., and Wells, H. G.: J. M. Research **30**:417, 1914.
Stahr, H.: Verhandl. d. deutsch. path. Gesellsch. **19**:188, 1923.
Sweany, H. C.: Arch. Path. **19**:203, 1935.
Uspensky, A.: Röntgenpraxis **9**:38, 1937.
Watanabe, Y.: Gann **33**:286, 1939.
Weissmann, S.: Frankfurt. Ztschr. f. Path. **47**:534, 1935.
Winternitz, M. C.; Smith, G. H., and McNamara, F. P.: J. Exper. Med. **32**:205, 1920.
Wohlwill, F.: Schweiz. med. Wchnschr. **68**:1186, 1938.
Womack, N. A., and Graham, E. A.: Arch. Path. **26**:165, 1938.
Young, J. S.: J. Path. & Bact. **31**:705, 1928.

# Book Reviews

**Survey of Compounds Which Have Been Tested for Carcinogenic Activity.** Jonathan L. Hartwell, associate chemist, National Cancer Institute, National Institute of Health, United States Public Health Service. Pp. 371 (multilithed). Price: available for free distribution. Washington, D. C.: Federal Security Agency, 1941.

Here collected for the first time in one volume are all the pertinent data on 696 chemical compounds which had been tested in animals for carcinogenic potency up to the beginning of the year 1940. From the various papers reviewed, the important facts have been abstracted, with the purpose of obtaining a comprehensive survey of a single limited field—that of chemical carcinogenesis. Included are some as yet unpublished material from the National Cancer Institute and much that has hitherto been virtually inaccessible in obscure foreign papers, which the author has consulted either in the original or in translation.

The volume is arranged in three parts. An introduction together with a note on the scope of the work and chemical considerations covers eleven pages. In the chapter on chemical considerations the author discusses the nomenclature of the compounds commonly employed in the ever enlarging field of experimental cancer research—a discussion which will be of particular value to new investigators. The main body of the work is arranged in tabular form, covering 284 pages; the material is grouped according to chemical compounds. Under each compound, the pertinent data are listed in ten categories, which include the reference, the animal tested together with its sex and strain, the preparation and dose of the compound, the site or the route of administration, the number of animals with induced tumors, the period of survival of the animal, the duration of the experiment and remarks.

Only single chemical compounds are considered. Such complex mixtures as the tars derived from a variety of materials, the irradiated sterols and the articles of diet are excluded. Likewise, mixtures of two or more pure compounds given simultaneously are not covered. Generally included, however, are crude grades of definite chemical individuals. The guiding principle has been to consider only such mixtures as render it feasible to determine to which constituent the given effects are attributed. Both inorganic and organic compounds are treated, and radioactive elements are also included since, although their action may be generally due to physical radiation, in many cases the element is applied directly to the tissue and the possibility of chemical action cannot be rejected.

The author emphasizes the incompleteness of the data on most of the compounds listed, with the consequent limitation that must be imposed on deductions drawn from them, and enters a plea for the publication of more complete data in the future, doubly necessary in long term work, the repetition of which by other laboratories is so costly. The most obvious needs are for more complete investigation of compounds which have already been studied in a preliminary fashion and for filling large gaps in the list of new substances which it would seem desirable to test. As an example, the field of the biologically occurring steroids is cited as hardly opened, although experiments of long duration have shown that certain members of this group, notably the estrogens, induce tumors locally at the sites of injection and in distant organs. Considering that these compounds occur in nature, an inquiry into the structural features responsible for their carcinogenic properties would appear to offer considerable promise of a greater understanding of the causes of some types of cancer in man.

The author points out that in the United States there is an increasing amount of research being carried out on the numerous general problems depending on chemical carcinogenesis, particularly the correlation of carcinogenic activity with chemical structure. It is impressive that the 192 compounds which have been

proved actively carcinogenic are found in a great variety of chemical classes and that substances of widely differing chemical structure possess similar carcinogenic activity; whereas, on the other hand, of two given chemical substances of the closest structural similarity, one may and the other may not be active. These considerations at first thought may make it seem hopeless to connect carcinogenicity in any way with chemical structure. However, it is the author's opinion that there is no justification for relaxing efforts in this direction of approach. Indications of what points should be revealed by studies along this line are the already growing ideas of organ and tissue specificity and species selectivity among certain carcinogenic compounds. Further careful studies of previously inadequately tested compounds and of compounds not yet employed may reasonably be expected to throw light on the causes of different types of cancer.

The monograph is rendered easily usable by the inclusion of four indexes, which cover, respectively, the route and the site of application, the species of animal tested, the site of tumor development and the vehicle in which the carcinogenic agent is carried. Finally there are also a compound index and a bibliography.

**Diabetes Mellitus.** Zolton T. Wirtschafter, M.D., clinician in charge, Clinic for Diabetes, Department of Medicine, Mount Sinai Hospital, Cleveland; visiting physician, Department of Medicine, Cleveland City Hospital; clinical instructor in medicine, School of Medicine, Western Reserve University, Cleveland; and Morton Korenberg, M.D., former fellow, May Institute of Medical Research, Jewish Hospital, Cincinnati; medical resident, Jewish General Hospital, Montreal. Cloth. Pp. 186, with 9 figures and 3 tables. Price, $2.50. Baltimore: The Williams & Wilkins Company, 1942.

This book represents a courageous attempt to compress within its 160 pages of text the essence of present knowledge relating to the pathologic physiology and clinical aspects of diabetes mellitus. The chapter headings are: "History," "Insulin," "Glycogenesis and Glycogenolysis," "Ketosis," "Phosphorus," "Acid-Base Equilibrium," "Glucose Tolerance Tests," "The Vitamins," "The Endocrines," "The Organ Systems," "Diabetes Mellitus" and "Diabetic Coma." Considerably more than half the contents are devoted to a discussion of theory and experimental evidence, and the physician chiefly interested in a comprehensive guide to diagnosis and treatment will want to supplement his information from other sources. Appended are an index and a bibliography of 336 references, most of which are taken from the literature of the past five years.

Attention must be called to certain faults. The authors recognize that "the numerous theories and diverse methods of treatment have left both the student and the practitioner hopelessly bewildered." Their method of bringing order out of chaos, however, is to confine themselves uncritically and almost completely to one school of thought, that represented by Soskin and Mirsky, who are so freely quoted as nearly to have earned the title of co-authors. The emphasis throughout is on the all-importance of hepatic gluconeogenesis, glycogenesis and glycogenolysis. It is these functions that insulin controls, and the reader unfamiliar with the field would not guess that this hormone has even been suspected of having anything to do with carbohydrate oxidation. Similarly, with respect to treatment, no mention is made of any other than a high carbohydrate diet. This, together with the unqualified recommendations that no diet should contain more than 100 Gm. of fat and that the calories should be divided in the ratio of 1:2:2:1 between four meals, hardly bears out the claim of the publishers that therapy is individualized and "the pitfalls and dangers of a mechanical approach to the problem are emphasized."

Certain of the material appearing, as it must in this subject, in more than one chapter is rediscussed, often verbatim, as if for the first time. Such repetition is particularly unfortunate in a monograph whose size bespeaks the intent of brevity. Parts of the chapter on organ systems, while interesting, are not

strictly relevant. Descriptions of the duration of action of the various types of insulin are given without properly defining the conditions under which the facts were obtained. Thus, the flat statement that "maximum hypoglycemia occurs between the tenth and twelfth hour following injection of protamine zinc insulin" (pages 7 and 137) is true only for a single dose given to a fasting subject and not for the daily doses used in the treatment of the diabetic patient. Loose thinking such as the following appears in several places: "Hyperpituitarism causes a stimulation of hepatic glyconeogenesis. . . ." (page 22). Hyperpituitarism is a clinical term, and no one knows what happens to the liver glycogen of such a patient. The authors doubtless are referring to the effect of anterior pituitary extracts in animals, but they do not say so.

The book should not be condemned because of its shortcomings. It contains a great deal of up-to-date information and provides a stimulating summary of certain modern trends of thought in the field of carbohydrate metabolism. The reviewer cannot help wishing that the volume were so entitled.

**The 1941 Year Book of Pathology and Immunology.** Pathology edited by Howard T. Karsner, M.D., professor of pathology, director of the Western Reserve University Institute of Pathology, Cleveland; Immunology edited by Sanford B. Hooker, A.M., M.D., professor of immunology, Boston University School of Medicine; member, Evans Memorial for Clinical Research and Preventive Medicine; immunologist, Massachusetts Memorial Hospitals. Pp. 623. Price $3. Chicago: The Year Book Publishers, 1941.

This is the second issue of the "Year Book of Pathology and Immunology." It covers the available periodic literature in those sciences, now limited almost wholly to the English language, for the year ending approximately with October 1941. The part devoted to pathology occupies 344 pages and is divided into 12 chapters: general pathology; tumors; cardiovascular system; hemopoietic system; respiratory system; alimentary tract and associated glands; urinary system and male genitalia; female genitalia and breast; organs of locomotion; glands of internal secretion; nervous system, and technical methods. There are 129 illustrations, all copied from the articles abstracted. The part on immunology takes up 344 pages and reviews: immunity to bacterial diseases; immunity to viral and rickettsial diseases; immunity to protozoa, fungi and higher parasites; immunity to tumors; chemotherapy; anaphylaxis and allergy; blood groups; immunochemistry; bacteriology and bacterial nutrition; technics, and miscellaneous topics. There is a good subject index, as well as an index to authors. The publisher's work is well done. The book gives good, well selected summaries—bird's eye views—of the developments in its fields for the period in question.

**The March of Medicine.** New York Academy of Medicine, Lectures to the Laity, 1941. Pp. 154. Price $2. New York, Morningside Heights: Columbia University Press, 1941.

For some years the New York Academy of Medicine has conducted each year a series of lectures for the laity. The general purpose of these lectures is to consider the social and cultural significance of medicine on a historical background. The present book, the third of its kind, contains the lectures for 1941: "Humanism and Science," Alan Gregg; "Paracelsus in the Light of Four Hundred Years," Henry E. Sigerist; "Psychiatry and Normal Life," William Healy; "Philosophy as Therapy," Irwin Erdman; "The Promise of Endocrinology," Oscar Riddle; "What We Do Know About Cancer," Francis Carter Wood. The range of topics is noteworthy and each of the lecture-essays is eminently good in its field. The book will interest persons of understanding no matter what their calling may be.